Attorney Docket No.: 38530-0100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:
Sarah Anne Robertson *et al.*

Confirmation No.: 2475

Application No.: 09/380,327

Art Unit: 1644

Filed: September 3, 1999

Examiner: Michail A. Belyavskyi

For: **TREATMENT AND DIAGNOSIS OF INFERTILITY USING TGFβ OR ACTIVIN**

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. John C. Herr, PhD. being duly warned, hereby declare and say:

1. I am Director of the Center for Research in Contraceptive and Reproductive Health at the University of Virginia, Charlottesville, VA.

2. I have a PhD from the University of Iowa.

3. I am a past President of the American Society for Reproductive Immunology. In 1994 I was awarded the Blackwell Munskgaard Senior Investigator Award from the American Society for Reproductive Immunology, which is presented annually to a senior investigator who has made outstanding contributions to the area of reproductive immunology.

4. I am the principal author of numerous articles related to immunological mechanisms of human infertility and reproductive immunology. My *curriculum vitae* is attached.

5. I am being compensated for preparing this declaration at my normal consulting rate.

6. I have reviewed the following documents:

The disclosure of U.S. Application Serial No. 09/380,327 ("the '327 application");

The Official Actions from the United States Patent Office dated October 6, 2004, March 24, 2005, and August 1, 2005;

U.S. Patent 5,395,825 (1995) - Feinberg *et al;*.

Lea *et al.* AJRI, 34:52-64 (1995);

Prakash, Reprod. Immunol., 35: 403-412 (1981);

Chapter 2: WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction (1987);

Harlow and Lane: A Laboratory Manual, page 61 (1988);

Martin-Villa *et al.*, Bio. Reprod., 55: 620-629 (1996);

Thaler, C., AJRI, 21: 147-150 (1989);

Grainger *et al.* Nature Med., 1,9: 932-937;

Thomas & McLean, AJRI, 6:185-189 (1984);

Nocera & Chu AJRI, 33: 282-291 (1995);

Heidenreich *et al.* AJRI, 31: 69-76 (1994);

Clark *et al.* Human Reprod., 9: 2270-2277 (1994).

7. I am informed that there is a two-part test for determining whether a claim is obvious. This test requires considering whether the prior art (i) would have suggested to one of ordinary skill in the art that the claimed invention should be carried out and (ii) would have had a reasonable likelihood of success, viewed in the light of the prior art.

8. I understand that the Examiner states that Feinberg teaches a method of treating infertility by administering TGFβ along with antigens such as sperm into the reproductive tract of a prospective mother. Allegedly, the TGFβ can be administered before, simultaneously with, or after, paternal antigens. Feinberg states that the TGFβ aids implantation of a conceptus by stimulating the production of trophoblast fibronectin by the conceptus.

9. One of ordinary skill in the art would have known that TGFβ has an *in vivo* half-life of only a few minutes. Wakefield *et al., J Clin Invest. 86:1976-84 (1990)(appended to this declaration as EXHIBIT 1);* Philipa *et al., J Biol Chem. 266:22290-6 (1991)(EXHIBIT 2).* In view of Feinberg's clearly stated objective of stimulating production of fibronectin by the

conceptus, one of ordinary skill in the art would have understood that Feinberg could only have contemplated increasing the success rate of assisted reproduction if TGFβ is delivered precisely at the time during which the preimplantation embryo arrives in the uterine cavity, and not a week or months before attempted conception. This is logical since Feinberg's methods are aimed at increasing fibronectin production in the conceptus – it would be pointless to administer TGFβ until a conceptus is present and able to respond to the TGFβ. Delivery of TGFβ at any other time prior to introduction of the conceptus would have no effect because the TGFβ would be degraded before it could contact the conceptus and have any effect on fibronectin production. Although Feinberg states that TGFβ and semen can be introduced into the maternal reproductive tract prior to introduction of the ovum, one of ordinary skill in the art would recognize that the time period between introduction of the TGFβ and the ovum would have to be on the order of minutes if there was to be any possibility for the TGFβ to stimulate fibronectin production.

10. Claim 105 of the '327 application requires that TGFβ be delivered at least one week before attempted conception, that is, at least one week before a conceptus possibly could be present in the prospective mother's reproductive tract.

11. Feinberg would not have provided any motivation to deliver TGFβ at least one week before attempted conception. Rather, because Feinberg states that the intended target of the TGFβ is the conceptus, the earliest time one would deliver the TGFβ would be immediately prior to the time of implantation, which is 4 days after conception.

12. I disagree with the Examiner's statement that it would have been obvious to treat recurrent miscarriage by inducing immune tolerance to a paternal antigen in a prospective mother. The Examiner argues that Prakash, Thaler *et al.* and Thomas *et al.* teach exposing the maternal genital mucosal surface to semen and that Thomas *et al.* teaches that semen contains an immunosuppressive factor, such as TGFβ, that suppresses postcoital T-cell response. The Examiner also states that Lea *et al.* teaches that patients suffering from recurrent spontaneous abortion are deficient in uterine TGFβ2-producing suppressor cells. Clark *et al.* is cited as teaching that TGFβ has immunosuppressive activity that leads to induction of tolerance *in* vivo during the first trimester of pregnancy in humans. Nocera *et al.* is cited as teaching that human seminal plasma contains a TGFβ precursor and Thaler is cited as teaching that human seminal

plasma contains factors that suppress the maternal immune response to paternal antigens and could prime mothers for improved implantation rates.

13. The experiments described by Lea *et al.* showed that TGFβ2-related molecules are released by decidual lymphoid cells and that the supernatant from these cells is able to suppress the *in vitro* immune activity of normal human blood lymphocytes (Figure 1, page 56) and suppress the generation of cytotoxic T lymphocytes using a mixed lymphocyte culture-cytotoxic lymphocyte (MLC-CTL) assay (Figure 3, page 58). The assay used by Lea *et al.* did not and could not show antigen-specific immune tolerance. At best the assay shows a generalised immunosuppression effect. Lea does not make any reference to immune tolerance. As a result, nothing in Lea *et al.* teaches or suggests that the cell supernatant can induce immune tolerance.

14. Similarly, the assay described by Nocera *et al.* did not and could not show antigen-specific immune tolerance. At best the assay shows a generalised immunosuppression effect. Nocera does not make any reference to immune tolerance. As a result, nothing in Nocera *et al.* teaches or suggests that the cell supernatant can induce immune tolerance.

15. Clark *et al.* describes that the TGFβ2-related molecule released from CD56+ cells obtained from decidua of human first trimester pregnancy can inhibit T lymphocyte generation in vitro (using the MLC-CTL assay). As with Lea *et al.* and Nocera *et al.*, the *in vitro* assay used by Clark *et al.* did not and could not show antigen-specific immune tolerance. At best the assay shows a generalised immunosuppression effect. Clark does not make any reference to immune tolerance. As a result, nothing in Clark *et al.* teaches or suggests that the cell supernatant can induce immune tolerance.

16. Thomas *et al.* states that seminal plasma has some undefined and unexplained immunosuppressive effect on the cellular immune reaction of the female to male antigens after mating. Thomas does not make any reference to immune tolerance. Nothing in Thomas *et al.* teaches or suggests that seminal plasma can induce immune tolerance.

17. Thaler *et al.* discusses the generalised immunosuppressive activities of seminal plasma. There is no suggestion of immune tolerance.

18. Prakash et al discusses the generalised immunosuppressive properties of semen. There is no suggestion of immune tolerance.

19. Generalized immunosuppression is the suppression of the immune system towards all antigens. This phenomenon is not antigen-specific. The induction of generalized immunosuppression is immediate, resulting in changes in the proliferative ability of lymphocytes that is detectable within hours. Generalized immunosuppression is also a temporary phenomenon because it is not memory related and can be reversed once the suppressive agent is removed. Generalized immunosuppression has potentially significant detrimental side effects because it can result in the immune system becoming non-functional, leaving the host vulnerable to infectious diseases and tumor proliferation.

20. By contrast, immune tolerance is not a suppression of the immune response towards all antigens (i.e. "generalized" immunosuppression), but is a conducive active immune response towards a specific antigen. Even when the immune system is tolerant to a particular antigen or antigens, it still is fully functional to other antigens and the host is not vulnerable to infectious diseases and tumour proliferation. Furthermore, the induction of immune tolerance requires a priming event, and involves a process of activation and expansion of immune mediating cells (T-lymphocytes) that proceeds over several days (generally at least 2-3 days after antigen administration, to allow for the necessary intermediate events of antigen processing and presentation, T-lymphocyte activation and proliferation) (Huang *et al, J. Immunol.*; 170:3945-3953 (2003)).

21. Generalized immune suppression is inconsistent with activation of immune tolerance since the priming, activation and expansion of the immune cells responsible for immune tolerance is inhibited by generalized immune suppression; induction of generalized immune suppression in a prospective mother would actually inhibit the induction of immune tolerance. Finally, immune tolerance is memory related and is long lasting, for at least as long as the activating antigen persists in the system, and immune tolerance established prior to conception lasts the entire period of pregnancy (Tafuri *et al., Science* 270:630-3 (1995)).

22. None of the assays described by Lea *et al.*, Nocera *et al.*, Clark *et al.*, Thomas *et al.*, Thaler *et al.*, or Prakash are capable of demonstrating any induction of immune tolerance. Moreover, none of the references, either alone or in combination, teach or suggest that TGFβ or semen, or the combination of TGFβ and semen, can induce tolerance in a prospective mother.

23. In summary, Feinberg states that TGFβ can increase the chance of successful implantation of a conceptus by stimulating fibronectin production in the conceptus. Feinberg

does not mention immune tolerance, nor suggest that TGFβ might play a role in the induction of tolerance. The other references (Lea *et al.*, Nocera *et al.*, Clark *et al.*, Thomas *et al.*, Thaler *et al.*, and Prakash) suggest at most that TGFβ present in semen may have some ill-defined local generalized immunosuppressive effect in a prospective mother, but fail to teach or suggest that exogenous TGFβ can be used in combination with paternal antigens to induce tolerance in the mother.

23. Finally, the invention defined by the claims of the '327 application has received wide recognition by peers in the reproductive immunology field because it provides a solution to an unmet need. This peer recognition is evidenced by the award of the 2005 J. Christian Herr Award to Dr. Sarah A. Robertson. I presented the Award to Dr. Robertson at the 25th Annual Meeting of the American Society for Reproductive Immunology in Providence, RI on June 16, 2005. The Herr Award is given to an individual who has made outstanding achievements in reproductive immunology research. Dr. Robertson's research explores the roles of cytokines in early pregnancy and specifically identified TGFβ in seminal plasma as an agent capable of inducing immune tolerance in the female reproductive tract. This latter discovery described a new paradigm for the role of male factor signalling in the events of early pregnancy, and led directly to the methods for the treatment of reproductive disorders described in the '327 application. In my opinion Dr. Robertson's research on seminal TGFβ was a significant reason for award of the J. Christian Herr Award.

24. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

3-16-06
Date

John C. Herr
John C. Herr, PhD

JOHN CHRISTIAN HERR

Curriculum Vitae

Current Status:	Professor of Cell Biology
	Professor of Urology
Other Titles:	Director of the Lymphocyte Culture Center
	Director of the Center for Research in Contraceptive and Reproductive Health
	Dean of School of Medicine's Special Assistant for Development

Address

Department of Cell Biology
University of Virginia Health System
School of Medicine P.O. Box 800732
Charlottesville, VA 22908-0732
Tel: (434) 924-2007
Fax: (434) 982-3912
E-Mail: jch7k@virginia.edu
http://hsc.virginia.edu/medicine/basic-sci/cellbio/crgcv/

Personal Data

Birthdate:	June 29, 1948
Birthplace:	Dubuque, Iowa
Citizenship:	U.S.; Ethnic Origin: Caucasian [Northern European]
U.S. Social Security Number:	480-56-3234
U.S. Passport #:	012851562 [expires August 8, 2006]
Marital Status:	Married to Mary Jo Herriman, two children:
	Christian Craig Herr (b. 6/23/83); Austin King Herr (b. 9/18/88)

Education

Summer Program:	1968	Hopkins Marine Station, Stanford University [Phycology (algae, plankton)]
B.A.	1970	Grinnell College, Grinnell, Iowa [General Biology, Education]
Ph.D.	1978	University of Iowa, Anatomy & Cell Biology [Advisor: P.M. Heidger]
Postdoctoral	1978-81	University of Washington, Seattle [Advisor: E.M. Eddy; reproductive immunology]

Academic Experience

1. 1974-1978	National Institutes of Health Trainee in Anatomy, University of Iowa, Dept. Anatomy.
2. 1978-1979	Fellow: NICHHD Interdisciplinary Training Program in Developmental Biology, University of Washington
3. 1979-1981	NIH National Research Service Award Postdoctoral Fellowship, University of Washington, Department of Biological Structure. "Sperm Surface Antigens"
4. 1981-1987	Assistant Professor, Department of Anatomy & Cell Biology, University of Virginia, Charlottesville, Virginia.
5. 1982-present	Director, University of Virginia Lymphocyte Culture Center.
6. 1987 -1992	Associate Professor Anatomy & Cell Biology, University of Virginia.
7. 1990-1996	Director, Biotechnology Training Program.
8. 1991-present	Director, NIH Center for Research in Contraceptive and Reproductive Health [formerly Center for Recombinant Gamete Contraceptive Vaccinogens].
9. 1992-present	Professor of Cell Biology, University of Virginia.
10. 1999-present	Dean's Special Assistant for Development
11. 2003-present	Professor of Urology

Professional Affiliations

Sigma Xi
American Association of Anatomists
American Society for Cell Biology
American Society for Reproductive Immunology
American Society of Andrology
Society for the Study of Reproduction
International Society for the Immunology of Reproduction

Areas of Current Interest

Cell biology of gametogenesis: specifically: differential gene expression during spermatogenesis and oogenesis. Reproductive immunology: postvasectomy autoimmunity. Applied research: contraceptive vaccine development; identification of contraceptive drug targets; infertility diagnostics; male contraception; spermicides; cancer and forensic biomarkers.

Service to Societies

2004-pres	Awards Committee, Am. Soc. Reprod. Immunology
2003-pres	Public Affairs Committee, American Society Andrology
2003	Program Committee, American Society of Andrology, 2005 Annual Meeting
2002	Program Committee, American Society Andrology, 2004 Annual Meeting
2001	Organizing Committee, Testis Workshop, Phoenix, AZ
2001	Councilor - American Society for Reproductive Immunology
1998-00	Councilor - International Society for Immunology of Reproduction

1998	Abstract Review Committee, Society for the Study of Reproduction
1996-97	Host, First International Conference on Experimental and Clinical Reproductive Immunobiology, October, 1997, Charlottesville, VA
1995-96	Organizing Committee, 1996 Meeting of American Society of Reproductive Immunology
1994-95	Organizing Committee, Sixth International Congress of Reproductive Immunology
1993	Organizing Committee, Serona Symposium, Immunology of Reproduction, August 26-29, 1993, Boston, MA
1993	Abstract Review Committee, Society for the Study of Reproduction
1993-94	President, American Society for Reproductive Immunology
1991	Abstract Review Committee, American Society of Andrology
1991	Organizer of the 11th Annual Meeting of the Am. Society for Immunology of Reproduction, Charlottesville, VA
1989-94	Councillor- American Society for Immunology of Reproduction
1988-91	Educational Affairs Committee - American Association of Anatomists
1987	Centennial Fund Raising Committee, American Association of Anatomists
1985-88	Committee member, Planning Committee for 1987 Centennial Meeting, American Association of Anatomists
1985	Committee member, Criteria for Admissions to AAA Directory
1985-86	Committee member, Nominations Committee, American Association of Anatomists
1984	Committee member, Committee to Improve the Annual Meeting, American Association of Anatomists
1983-86	Chairman, Advisory Committee of Young Anatomists, American Association of Anatomists

Awards

1995	Munksgaard Senior Investigator Award, American Society for Reproductive Immunology, Washington, D.C., July 22, 1995
1999	Christopher J. Henderson Inventor of the Year Award, University of Virginia Patent Foundation, April 26, 1999
2000	Virginia's Outstanding Scientist 2000 Award, March 27, 2000
2001	The Burroughs Wellcome Visiting Professorship in Reproductive Biology, University of Guelph, Ontario, Canada. Jan 29-Feb 2, 2001
2002	The Distinguished Alumnus Award for Achievement-University of Iowa, May 30, 2002
2004	The Raymond O. Berry Memorial Lectureship – Texas A&M University, April 2, 2004
2004	The Small Business Innovation Research Commercialization Breakthrough Award, Virginia Center for Innovative Technology, October 13, 2004 Arlington, VA

Editorial Boards

1986-1998	Anatomical Record
1988-2000	Journal of Reproductive Immunology
1991-1994, 1997-1998	American J. of Reproductive Immunology
2000-2004	Biology of Reproduction

Reviewer for Journals

PNAS
International J. of Cancer
International J. of Andrology
Journal of Cell Science
Journal of Cell Biology
Nature Biotechnology
Life Sciences
Journal of Histochemistry and Cytochemistry
Journal of Andrology
Reproduction, Fertility, Development
Biology of Reproduction
American Journal of Anatomy
Journal of Investigative Urology
Developmental Biology
Journal of Reproduction and Fertility
Journal of Urology
Biochemical Pharmacology
Molecular Human Reproduction
Journal of Immunology
Fertility and Sterility
Protein Expression and Purification
Journal of Clinical Investigation
Experimental Cell Research

Study Sections

2004 Review Intramural Program: Laboratory of Biosystems and Cancer, National Cancer Institute, September 19-21, 2004
2004, Special Emphasis Panel "NRSA Applications", March 10 and 11, 2004
2004, NIH Minority Biomedical Research Support Panel ad hoc reviewer, Feb. 10, 2004
2003, NIH Conference Panel: "Strategic Planning Meeting for the Fogarty International Center Programs" Dec. 12, 2003
2003, Chairman NIH Special Emphasis Panel: Synthesis and Testing of Nonsteroidal and Nonhormonal Male Contraceptive Agents, Sept. 17, 2003
2002, NIH Special Emphasis Panel, JWG, May 2002.
2002, NIH Special Emphasis Panel, NRSA Applications, March 5, 2002.
2002, NIH Special Emphasis Panel, JWG, May 2002.
2002, NIH Special Emphasis Panel, NRSA Applications, March 5, 2002.
2001, NIH Special Emphasis Panel, P30 Center, Washington State, July 24.
2001, NIH Special Emphasis Panel, February 19, Support Contracts for Contraceptive and Reproductive Health Branch [CRHB]
2000, NIH Special Emphasis Panel, June 27-28, P30 Center Washington State
2000, External Reviewer, Jeffress Memorial Trust
1999, Ad hoc Reviewer, SBIR Study Section, US Dept. Agriculture.
1999, External Reviewer for Royal Society of Great Britain.
1998 Member, National Institute of Allergy and Infectious Diseases Task Force on Immunology.
NIH Special Emphasis Panel Behavioral Medicine Study Section, Dec 11, 1998

External Reviewer for NIH Intramural Programs, Fall 1998
External Reviewer for Wellcome Trust, 1994, 1995, 1997, 1999, 2000
External Reviewer for Israel Science Foundation, 1997, 1998
External Reviewer for WHO Program in Reproductive Research and Training, 1995
Member, National Cancer Institute Special Study Section "Scalable GMP Production", April 22, 1994; October 4, 1994
Member, NIH-NICHD Special Study Section - P50 Center, Aug. 23-25, 1993
Member, NIH-NIGMS Biotechnology Training Programs Study Section and 2 Site Visit Teams, Fall 1993
Member, Immunocontraceptive Working Group, U.S.A.I.D., 1992-1993
Chairman, NIH Special Study Section, "Development of a Contraceptive Vaccine, October 9, 1992
Member, Molecular Immunology and Vaccine Development Panel of the National Task Force on the NIH Strategic Plan, June 23-25, 1992
Member, Expert Panel on Wildlife Damage and Population Regulation, U.S. Dept. Agriculture, April, 1992
Member, Immunocontraceptive Working Group, Contraceptive Research and Development Program, USAID, September, 1991
Member, NIH-NICHD Special Study Section-Program Project, "A Coordinated Program in Reproductive Biology," May, 1991
Member, United States Agency for International Development Contraceptive Research and Development Program Working Group, May, 1990
Member, NIH-NICHD Population Research Committee, Aug. 1990
Member, NIH - NIDDK Study Section, Program Project: "Experimental Models of Gene Therapy," December 11-13, 1989
Member, NIH - NIDDK Special Study Section, Small Business Innovative Research, Fall 1988
Member, NIH - NICHHD Special Study Section - Population Research Center, April 6-9, 1988
Ad hoc reviewer, NSF Developmental Biology Study Section

International Service

2004- Program Committee, "Advances and Challenges in Reproductive Health in the Post Genomic Era" held Jan 9-12, 2005, Mumbai, India.

In 1990, Dr. Herr received a grant from USAID to organize a course, "Molecular Biology of Human Spermatogenesis" which was taught in March of 1990 at the National Institute of Immunology in New Delhi, India. This 16 day course covered methods of cloning genes from the human testis and was attended by students, postdoctoral fellows and faculty members from universities throughout India. A fully functional rDNA laboratory was transported to New Delhi and was donated to the Indian government at the conclusion of the course. Dr. and Mrs. Richard Wright assisted in this effort.

In 1992, a grant from the NIH supported a workshop at the Institute for Chemical Biology in Calcutta, India. Dr. Herr's lab organized a two-week course, including Dr. Herr, Ken Klotz, and Alex Freemerman, also from Dr. Herr's laboratory. The course covered basic methods of molecular biology including RNA isolation and cDNA library construction. Laboratory equipment for molecular biology training was assembled in Virginia for the course and donated to the Institute at the conclusion of the course.

In 1995, Dr. Herr served as a visiting professor in Cairo, Egypt where he lectured at Ain Shaims University, Al-Azhar University, and Cairo University and visited the Marine Biology Station at Suez. The topics of his lectures and discussions were the trends and opportunities in contraceptive vaccine research and development.

In 1998, Dr. Herr organized a team to teach a laboratory in the Frontiers in Reproduction Course at Woods Hole, Mass. The course was attended by 16 international scholars in 1998 and was again taught in 1999.

In 2000, Dr. Herr was appointed as a member of the Indo-US Joint Working Group comprised of members from the NIH, State Department, USAID and U.S. scientists as well as Indian counterparts in government and academics. This group fosters collaborative scientific efforts between the two countries and reviews bilateral grant applications.

Boards of Directors of Corporations and Foundations

1983-1987	Humagen, Inc
1992-Present	ContraVac, Inc
1998-2008	University of Virginia Patents Foundation
2005	Translational Research Partnership with the Wallace H. Coulter Foundation

University Committees Served

2004 [Fall]	Chair Review Committee, Department of Obstetrics/Gynecology
2004-2005	CRCRH Faculty Search Committee, [Chairman]
2004	Department of Urology Faculty Search Committee
2004-2005	School of Medicine Search Committee for Associate Dean for Clinical Research
2002-2003	School of Medicine Resources Committee
2002-2004	UVA Technology Commercialization Working Group (Provost's Office)-Leadership sub-committee; Infrastructure sub-committee
2002-	Chairman, Henderson Inventor of the Year Committee
2001-present	Faculty Development Council
2000-2001	Search Committee, Development Office, UVA Health System, Director of Development for Immunology Research
2000-2001	Search Committee, Dept. Cell Biology, Faculty Replacement
1999-present	Medical Advisory Council to Dean School of Medicine
1999-present	UVA Research Parks Communications Council
1999-present	Health Sciences Corporate and Foundation Relations Advisory Council
1999-2001	Science and Technology 2020 Planning Commission
1998-present	Chairman, Histology Research Core Advisory Committee
1998-present	Library Capital Campaign Committee
1998-present	Chairman, Faculty Advisory Committee-Univ. of Virginia Patent Foundation
1997-1998	LCME Accreditation Task Force
1996-1998	Dean's Task Force on US News Survey of Medical Schools
1991-2001	Faculty Task Force on the School of Medicine Capital Campaign
1994-1998	Faculty Task Force for Biomedical Research
1994-1995	Institutional Research Policy Advisory Committee

1994-1997	Search Committee for University/Industry Research Coordinator, Office of Vice Provost for Research
1994-1995	Chairman, Faculty Forum for Scientific Research
1992-2001	Executive Committee Molecular Medicine Training Program
1993-1995	Member, Faculty Forum for Scientific Research
1991-1992	Committee for P-30 Grant for Reproductive Center Cores
1991-1997	Selection Committee for the University of Virginia Inventor of the Year Award [Dr. Richard Edlich, chair]
1991-1996	Graduate Advisors Committee
1990-1996	Executive Committee, Program Director, Biotechnology Training Program
1982-present	Committee for Lymphocyte Culture Core
1983-1988	Committee for FACS
1982-1983	Committee for Reproductive Biology Program Project (Gene Oliphant, Chairman)
1982	Committee for Organizing MAC Grant
1983-1986	Preclinical Committee (J. David Deck, Chairman)
1984-1988	Cancer Center Organizing Committee Program Project for Cancer Research Unit (R. Wagner - T. Parsons)
1987-1988	Committee for Reproductive Biology Training Grant
1987-1993	Search Committee, Department of Anatomy and Cell Biology
1985-1990	Gynecologic Cancer Committee
1989	Jefferson Award Committee (Department of Continuing Education)

Community Service

1999-pres	Biotechnology Curriculum Advisory Committee of Piedmont Virginia Community College
2000-2001	Founding member and Mate, Ship 19, Sea Scouts, Boy Scouts of America

In Training Teaching Activities

1975 Gross Anatomy for Medical Students, University of Iowa
1976 Principles of Human Anatomy for Nurses, University of Iowa
1976 Neuroanatomy, University of Iowa
1978 Histology for Medical Students, Univ. of Washington
1979 Musculoskeletal System, University of Washington
1979 Gross Anatomy for Dental Students, University of Washington

Overview of Teaching Activities at UVA

1981-82	Gross Anatomy for medical students; Course director: Anat. 506 Experimental Morphology
1982-83	Anat. 805 Special Topics in Dev. Anatomy Gross Anatomy for medical students; Course director: Anat. 506; Experimental Morphology, Biol 495, Anat 999.
1983-84	Gross Anatomy for medical students; Course director: Anat. 506 Experimental Morphology, Anat. 999

1984-85	Gross Anatomy for medical students; Director, Anat. 605 Experimental Methods; Anat. 595; Anat. 599; participant, Anat. 802 (Reprod. Biology), Anat. 999, Biol 495
1985-86	Gross Anatomy for medical students; Course Director, Anat. 805 Topics in Developmental Biology, participant in Anatomy 605, Anat. 999
1986-87	Gross Anatomy for medical students; participant in Anatomy 605, Anat. 999
1987-88	Gross Anatomy for medical students; Experimental Methods (Anat. 605); Advances in Reproductive Biology (Anat. 802), Anat. 999, Biol 495
1988-89	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1989-90	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1990-91	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1991-92	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1992-93	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1993-94	Gross Anatomy for medical students, Experimental Methods, Anat. 999
1994	Anatomy 805 Advances in Reproductive Biology
1994	Research Ethics GSAS 710
1995-98	Gross Anatomy for medical students
1998	Colloquium in Reproductive Biology-Gene Regulation of Spermatogenesis
2002	Gross Anatomy for medical students
2003	Gross Anatomy for medical students
2004	Gross Anatomy for medical students

Graduate Students Supervised and Supported (Thesis Advisor)

Years	Student	Positions following Ph.D.
1982-1987	Lisa Kisalus	Post-doctoral fellow: Harvard Medical School, Associate Director, Symposia, Serono, Inc.
1983-1987	Rob McGee	Post-doctoral fellow: NIEHS, Research Triangle, N.C. 1987-89. MD: Bowman-Grey 1990-1994. Residency - Pathology.
1985-1987	Nagiu Fares	Agency for Int. Dev. Peace Fellow from Egypt; conducted his thesis work with Dr. Herr. Currently Professor of Biology, Department of Biology, Faculty of Science, Ain Shams Univ., Cairo, Egypt.
1985-1989	Hal Handley	Post-doctoral fellow: Scripps Inst., La Jolla, CA. Currently Senior Scientist in biotechnology company-Maxim, Inc.
1987-1992	James Foster	Post-doctoral fellow: Univ. Pennsylvania, Dept. Ob-Gyn. Instructor, Haverford College. Currently Assistant Professor, Dept Biology, Randolf-Macon College, Ashland, VA.

1987-1991	Ming Chen Shen	Marriage and children.
1990-1992	Kelly Beecher	M.S. [1992], Research Assistant, Dept. Neuroscience, Univ. Kansas
1990-1994	Alex Freemerman	Postdoctoral fellow, cancer biology, Medical College of Virginia. Research Associate, Univ. Arizona. Currently Research Assistant Professor, Duke University, Dept. Surgery.
Began 1991	Cecelia Videus	Ph.D, Aug 1998: Winner Young Investigator Award; Am. Soc.Reprod. Immunol, 1996; entered Darden Business School Fall 1998. Summer internship 1999 at Pfizer. MBA, 2000. Currently investment banker specializing in biotechnology.
Began 1994	Lisa Norton	Ph.D, Aug 2000. Received 1998 Langman Award from Am. Soc. Anatomists for best grad student paper presented at FASEB meeting. Received 1999 Michael J. Peach Award for Outstanding Graduate Student, University of Virginia. Application Scientist, Combi Matrix, Seattle, WA.
Began 1999	Theresa Thompkins	Received 5 year NIH Predoctoral Fellowship for Minority Students for her MD/PHD. In the Summer of 2000 she received a National Merit Award.

Postdoctoral Fellows

7/1/83-6/30/84	Mark Sigman, M.D., Currently Professor of Urology, Brown Univ.
7/1/84-6/30/85	Bob Evans, M.D., Urology Resident - Dr. Evans' work with Dr. Herr was presented at the Mid Atlantic Urology Meetings on October 3, 1985 in Philadelphia where Dr. Evans won lst place for the best research paper presented by a Urology Fellow. He later went on to win second place at the National Urology Meetings in 1986. Dr. Evans' paper also won the Annual Medical Society of Virginia House Officer Prize for 1986. Dr. Evans is currently in private practice.
5/1/85-8/31/86	Nana Hussein Riad, Ph.D.- Professor and Chairman of Biology, Ains Shams University, Cairo, Egypt. Received Fullbright Scholarship to work with Dr Herr. Dr. Riad came to learn immunocytochemical procedures at light and EM levels. Dr. Riad returned in 1998 to UVA with a second Fullbright scholarship to learn molecular biology techniques.

5/1/86-7/31/86	Madhu Joshi, Ph.D. - Busch Scholar on Sabbatical, Professor of Anatomy, University of North Dakota, Fargo, N.D. Dr. Joshi came to Dr. Herr's lab to learn hybridoma techniques.
9/1/88-12/31/94	Mona Homyk, Ph.D. - subsequently worked for Humagen Fertility Diagnostics, Toxicology Regulatory Services, and currently, Pharmaceutical Research Associates.
7/1/88-93	Richard Wright, Ph.D. completed a DVM in 1998 at Virginia Tech and is practicing veterinary medicine in private practice.
3/1/89-6/30/03	Barbara Kurth, Ph.D., Senior Scientist, Associate Director Primate Models Core, Center for Research in Contraceptive and Reproductive Health.
7/1/90-6/31/91	David Mburu, DVM, WHO sponsored fellowship in molecular biology - postdoc in Australia.
7/1/90-8/31/91	Anil Suri, Ph.D., U.S.A.I.D. sponsored fellowship in spermato- genesis. Current position, Associate Professor, Natl. Institute Immunology, New Delhi, India. Dr. Suri has received USAID funding to return to Dr. Herr's lab for joint research in subsequent years. Dr. Herr currently supports a joint research project through his Mellon twinning grant. In 1999 Dr. Herr and Dr. Suri received a grant from the Indo-US Program. Dr. Suri returns to the Herr lab for a 2 month sabbatical each year.
9/1/90-8/31/91	Linsong Li, M.D., Postdoctoral Fellowship-University of Washington Department of Medicine.
10/5/92-6/31/95	Prabhakara Reddi. In 1992, Dr. Reddi was awarded a two-year Rockefeller Fellowship to work with Dr. Herr. He currently serves as Associate Director of the Bioprocess Core in Dr. Herr's Center and holds an appointment as Research Assistant Professor in the Department of Cell Biology. His NIH R29 grant received a 7.6% score and was funded in 1999 for 5 years. Dr. Herr also applied for and received a Mellon Foundation Junior Investigator Award for Dr. Reddi. In 2004 Dr. Reddi received an independent NIH R01 and a tenure track Assistant Professorship in the Department of Pathology at UVA.
10/5/92-1994	Dimpy Koul - U.S.A.I.D. CD&RI Fellow. Fogarty Fellow. Currently, post-doc at Baylor.
2/11/93-12/12/98	Soren Naaby-Hansen, M.D. Dr. Naaby-Hansen is an obstetrician who worked as a Mellon Foundation Fellow. He began an Asst. Professorship at the Ludwig Cancer Institute in London on January 1, 1999, where he continues collaboration with the Herr lab.
5/11/93-7/1/94	Zhang Jian, WHO Fellow. Returned to China to his previous Senior Scientist position.

6/15/93-6/30/03	Michael Wolkowicz, Ph.D - Mellon Foundation Fellowship. Instructor in Cell Biology. Head, Molecular Biology Group, Center for Research in Contraceptive and Reproductive Health.
9/15/93-2/28/97	Milena Mihailova, Ph.D - NIH Fellow. Not currently employed due to illness. Returned to Bulgaria, her home country.
6/1/94-4/17/02	Alan Diekman, Ph.D - NRSA Awardee, subsequently received NIH SBIR grant, Became Res. Assistant Professor of Cell Biology. NIH R01 received 0.9% percentile and 5 years of funding beginning on August 1, 1998. Currently, Assistant Professor of Biochemistry and Molecular Biology, Univ. of Arkansas Medical School, May 1, 2002.
7/1/94-6/30/02	Anne Westbrook-Case, Ph.D - NIH Traineeship, Mellon Fellowship, Berlex Fellow. Research Associate, Center for Research in Contraceptive and Reproductive Health. Currently: Senior Informative Scientist, National Library of Medicine, NCBI, NIH June 17, 2002.
1/28/95-4/1/03	Scott Coonrod, Ph.D - NIH Postdoctoral Fellowship. Dr. Coonrod held a Research Assistant Professor position in Cell Biology at the University of Virginia from 2001-2003. He received a 10% score on a NIH R01 grant which was funded December 1, 1999 for 5 years. On April 1, 2003 Dr. Coonrod began as an Assistant Professor of Genetic Medicine, Cornell Weill Medical School, NY., NY.
4/23/95-5/1/96	Vrinda Khole, Ph.D - WHO Fellowship - Dr. Khole and Dr. Herr subsequently collaborated under a WHO grant to Dr. Khole. Dr. Khole is Associate Professor at the Institute of Science, Bombay. Dr. Khole returned to Dr. Herr's lab in January 2000 for a 1 year sabbatical supported by the Fogarty International Center at NIH. Dr. Khole received a Mellon Twinning Grant from CONRAD in 2001. She had a two month sabbatical in the Herr lab in 2002 (Fall). In 2003 she was appointed deputy Director of the Institute for Research in Reproductive Health in Bombay.
7/1/95-8/1/96	Li Bin, Ph.D - PAN AM Health Organization Fellow specializing in chemical engineering. He has returned to China, his home country.
9/1/96-12/31/97	Hiroaki Shibahara, M.D. - training was sponsored by the Japanese government. Returned to Assistant Professorship, Hyogo Medical College, Japan. Currently Associate Professor of Obstetrics and Gynecology, Jichi Medical School, Japan.
9/1/96-6/30/2003	Arabinda Mandal, Ph.D - Fogarty Fellowship, Berlex Fellowship. 2003 - Instructor in Cell Biology.
11/15/96-present	Jagathpala Shetty, Ph.D - Fogarty Fellowship. In 2003 advanced to Senior Scientist in cell Biology.

7/1/98 - 2000	Tod C. McCauley, Ph.D - Berlex Fellowship / NIH Postdoctoral Trainee. Currently Research Associate, University of Missouri.
7/23/98-10/1/01	Buer Sen, Ph.D - Fogarty Fellowship. Currently, Postdoctoral Fellow, Emory University.
9/1/98-02/01/02	Friederike Jayes, Ph.D - Berlex Fellowship. Currently: Scientist, National Institute Environmental Health Sciences, Research Triangle Park, NC.
10/1/99-present	Zhonglin Hao, MD, Ph.D - Berlex Fellowship. Currently: Resident, Dept. of Internal Medicine, Medical Center of Central Georgia
7/1/00-6/30/2002	Mike Coppola, Ph.D - Berlex Fellowship. Director of Research, ContraVac, Inc.
10/1/00-present	Young-Hwan Kim, Ph.D - Fogarty Fellowship.
2/1/03-present	Kula Nand Jha, Ph.D – Fogarty Fellowship.
4/1/01-present	Dr. Belen Herrero - Berlex Fellowship.
5/1/02-present	Pamela Schoppee, Ph.D – Berlex Fellow
6/1/02-present	Kim Fralix, Ph.D - NIH Postdoctoral Trainee 1992-1993. In 2003 (June 1) Dr Fralix received a LALOR Fellowship.
1/9/02-present	Silvia Pullido, Ph.D – Berlex Fellow
5/1/03-present	BingFang Xu, Ph.D – NIH Fellow
7/1/03-present	Geeta Vanage, Ph.D – Fogarty Fellow- Associate Professor, National Instutute for Research in Reproductive Health, Mumbai, India
9/1/03-2/29/04	Alina Domagala, Ph.D – Fulbright Fellow- Researcher, Polish Academy of Sciences
9/1/03-present	Susan Sleight – Berlex Fellow
9/1/03-present	Jayasimha Rao – Fogarty Fellow
4/04-present	Sandeep Ranpura – Fogarty Fellow
1/26/04-present	Wei He, MD., Ph.D – Fogarty Fellow
4/01/04-3/30/05	Dr. Mangeet Sharma-Fogarty Fellow
4/01/04-present	Dr. Monica Sachedev-Fogarty Fellow

11/07/04-present	Yanfeng Li, MD, Ph.D.-Fogarty Fellow
10/07/04	Christian Gaudreault, Ph.D., CRCRH Fellow

Competitive Grants Awarded Previously

1979-81	NIH Postdoctoral Fellowship - "Isolation of Sperm Surface Antigens" NRSA
1981	University of Virginia Biomedical Research Support Award #5 S07RR 05431-20, $9,950 start-up funds: "Sperm Isoantigens Recognized by Monoclonal Antibodies"
1981	American Cancer Society Small Institutional Grant Committee, $5,000, pilot funds for "Anti-Sperm Antibody Production from Human X Human Hybridomas"
1982-present	Pratt Endowment Support for Establishing and Maintaining the Lymphocyte Culture Center
1982-85	Principal Investigator, NIH 1 R01 HD16767-01->03, "Antisperm Monoclonal Antibodies Isolated Post-Vasectomy." Project period: 9/30/82-9/29/85, $155,004 Direct Cost
1983-86	Principal Investigator, NIH 1 R01 HD17489 "Protein Synthesis and Secretion by Human Decidual Cells." Project Period: 4/1/83 - 10/30/86; $107,495 Direct Cost
1983-84	Principal Investigator, FBI Contract 115744 "Monoclonal Antibodies in Forensic Diagnosis," 10/1/83 - 9/3l/84. Direct Cost: $60,000
1983-86	Co-Investigator, NSF PC-8309364 "Secretion in the Epididymis" 12/83-11/86, $205,000, Dr. Charles Flickinger, P.I.
1984-90	Co-Investigator, NIH HD18825; "Vasovasostomy: Morphology, Physiology and Immunology," 8/1/84 - 9/31/90. Total direct cost: $311,053, Dr. Stuart Howards, P.I.
1984	Principal Investigator - Endotronics Industrial Collaborative Project, $130,000 industrial contribution - Acusyst 150, MMCM
1984-87	Co-Investigator and Principal Investigator (1985-86) NIH HD-12335 "Developmental Aspects of Mammalian Calcium Transport", 12/1/84-11/30/87, $233,531 Direct Cost, Dr. Elizabeth Bruns, Principal Investigator
1985-87	Collaborator - "Diabetes in the B.B. Rat" (NIH). Dr. Herr supervised one 1/2 time technician and one 3/4 time technician and directed histological aspects of this study. David Benjamin - P.I.
1985-86	Principal Investigator FBI Grant "MHS-5 & HSA Monoclonals" 2/1/85-1/31/86 Direct Cost $89,274
1985-88	Principal Investigator NIH HD16767-04->06 "Monoclonal Antibodies to Human Sperm" $175,359 Direct Cost 9/29/85-8/3l/88
1986-88	Principal Investigator "Monoclonal Antibodies to the Tumor Inhibitor PB-1" Center for Innovative Technology/Philadelphia Biologicals $47,994 2/1/87-1/31/88
1986-88	Principal Investigator, FBI Contract, "Monoclonal Antibodies to Vaginal Secretions" $91,942 Direct Cost

1988-91	Principal Investigator, "Cloning and Purification of a Sperm Membrane Immunogen" 2/1/88-1/31/91, NIH HD23789 Direct Costs: $346,564
1987-89	Principal Investigator, "Molecular Biology of Human Spermatogenesis," 8/1/87-7/31/89, Center for Innovative Technology and Humagen, Inc. Direct Costs: $237,002
1987-91	Principal Investigator, "Optimization of Immunoglobulin Secretion from Mouse and Human Hybridomas," Technology Development Center, $204,754 Direct Costs
1987-90	Principal Investigator, "Purification of Recombinant Immunogen and Scale Up of Expression System," Technology Development Center $88,670 Direct Costs
1988-89	Principal Investigator, "Toward Development of a Monoclonal Antibody Derivatized Sperm Cell Affinity Bead", Lifecodes Corp. $44,193 Direct Costs, 9/1/88-4/1/89
1988	Principal Investigator, "Scale-up of Hybridomas" Flow Laboratories $23,000 Industrial Equipment Donation
1988-90	Principal Investigator, C.J. Flickinger Co-Principal Investigator, "Localization of a Human Sperm Contraceptive Vaccine Immunogen", Mellon Foundation $85,000 11/1/88-4/31/90
1989	Principal Investigator, "World Health Organization Sperm Workshop", $11,000 11/1/89 - WHO
1990	Principal Investigator, "Workshop: Cloning and Sequencing Human Testicular Genes" CONRAD/U.S. A.I.D. $30,000
1991-1992	Principal Investigator, "Infertility Testing Under GLP", Ortho Pharmaceuticals, total direct costs 1/1/91-9/18/92, $316,050
1991-1994	Co-Investigator, "Vasovasostomy: Morphology, Physiology and Immunology", Stuart Howards, PI, NIH HD18825, 4/1/91->3/31/95, $344,549 total direct costs
1990-1995	Principal Investigator/Program Director, "Multidisciplinary Training Program in Biotechnology" NIH GM08401 $482,798 total direct costs, 9/1/90->6/30/95
1991-1994	Principal Investigator, "Baboon Infertility Testing of Recombinant SP-10 Vaccine" NIH HD 23789-04-06, 8/1/91->7/31/94, $301,639 total direct costs
1991-1994	Principal Investigator, "Incidence of SP-10 Antibodies in Sera and Secretions of Infertile Couples". United States Agency for International Development $237,636 direct costs, 10/1/91-12/31/93
1992-1996	Principal Investigator, "Interdisciplinary Training in Contraceptive Vaccine Development" Mellon Foundation, $700,000 1/1/93-4/1/96
1993-1995	Principal Investigator, "Baboon Fertility Trials of Recombinant Baboon SP-10", CONRAD, $301,746 direct costs, 9/1/93-2/28/96
1996-1997	Principal Investigator, "Postdoctoral Fellowship on Behalf of A.B. Diekman", NIH, $28,600 direct costs, 6/1/96-5/31/97
1991-2002	Principal Investigator, "Center for Recombinant Gamete Contraceptive Vaccinogens" NIH U54-HD29099, $6,789,297 direct costs, 9/1/91 - 8/31/96; 9/1/96 - 2/28/97, $713,849 direct cost; 3/1/97 - 2/30/98, $1,863,707 total direct cost; 3/1/98 - 2/29/99, $1,896,955 total direct cost; 3/1/99 - 2/28/00, $1,674,101 total direct cost; 3/1/00 - 2/28/01, $1,510,254 total direct cost.

1995-2000	Principal Investigator, D43 TW/HD00654 "Translational Contraceptive Research for Indian Postdocs", NIH Fogarty Center, 9/30/95 - 9/29/00 $1,069,952 total direct cost
1996-1999	Principal Investigator, "Multi-Institutional Interdisciplinary Postdoctoral Training", Mellon Foundation, $550,000, 4/1/96-3/31/99
1997-1998	Principal Investigator, ContraVac, "Recombinant Antibodies as Intra-Vaginal Spermicides," $9,200 direct costs, 10/1/97-3/31/99
1997-1999	Principal Investigator, CIT, "Recombinant Antibodies as Intra-vaginal Spermicide", $20,000 total direct cost, 11/1/97 - 10/31/99
1996-2001	Principal Investigator, "Isolation and Characterization of Genes Encoding Sperm Surface Antigens by Screening Human Testis Expression cDNA Library: Identification of a Candidate Molecule(s) for Development of Contraceptive Vaccine," Conrad MFG-96-19, $113,606 total costs, current year $27,018 total direct cost 10/1/96-09/30/01
1999-2002	Principal Investigator, "Training and New Research Initiatives in Contraceptive Development", Mellon Foundation, $250,000 total direct costs, current year, 4.1.99-3.31.02
1999-2002	Principal Investigator, "Carbohydrate Immunocontraceptive Epitopes Identified in a Neoglycolip Library Derived from the Human SpermGlycocalyx", Mellon Foundation, Junior Investigator Award on behalf of Alan B. Diekman $360,000 total direct cost current year, 4.1.99-3.31.02
1998-2002	Co-Investigator, "Cellular Regulation of the Developing Testis Following Vasal Ligation", NIH DK45179-01 $98,953 direct costs current year, 7/1/98-6/30/02

Current Funding

1998-2004	Principal Investigator "Program of Research and Development for Sperm Antigen" $4,507,530 total direct cost, $1,210,645 annual direct cost, Sponsor: Schering, AG
1998-2004	Principal Investigator, NIH HD35523 "Immunological Mechanisms in Human Female Infertility", $970,474 direct costs, 7/1/98-7/31/2004
2000-2004	Co-Investigator, "Characterization of Protein Tyrosine Phosphorylation during Human Sperm Capacitation", Mellon Foundation, $420,000 total direct cost, current year, 4.1.00-3/31/2004
2000-2005	Co-Investigator, NIH HD38353 "Oolemmal Proteomics", $214,062 total direct cost, current year, 2/1/00-1/30/05
2000-2005	Principal Investigator, NIH, "Research Training in Reproduction for Asian Fellows", $1,152,189 total direct cost, current year 9/30/00 - 9/25/05
2001-2004	Principal Investigator, "Contraceptive Vaccines for Dogs and Cats Based on Egg Membrane Antigens", The Kenneth A. Scott Charitable Trust, $62,300 total direct cost, year 1.
2001-2006	Principal Investigator, "Contraceptive Vaccines for Dogs and Cats Based on Egg Membrane Antigens". Genetics Savings and Clone, $775,520 total direct cost; $96,200 total direct cost, year 1.
2000-2003	Principal Investigator, "Sperm Binding Magnetic Beads for Forensic PCR/DNA Analysis", National Institute of Justice, $206,440 total direct cost, current year 10/1/2000-12/31/2003

2000-2004	Principal Investigator, NIH U54-HD29099 "Center for Recombinant Gamete Contraceptive Vaccinogens" $1,723,054 total direct cost, 3/1/01 - 2/29/04
2001-2004	Principal Investigator, "Spermatid Specific transcription factors as targets for novel male contraceptives." $420,000 total direct cost, Andrew W. Mellon Foundation Junior Investigator Award on behalf of P. P. Reddi
2002-2004	Principal Investigator, "Training and Research Infrastructure in Contraceptive Development." $250,000 total direct cost, Andrew W. Mellon Foundation
2003-2008	Co-Investigator, "Cellular Regulation in Genitourinary Development" Project 4: development and regulation of Antimicrobial Peptides in the GU Tract. $150,000.00 total direct cost, current year, 7/1/2003-6/30/2008 NIH
2004–2009	Co-Investigator, "Antimicrobial Proteins Secreted by the Epididymis" $180,000 total direct cost, 5/15/2004 – 2/28/2009.

Preceptor on Training Grants and Program Projects

1982-present	Participant in the Developmental Biology Training Grants and the Cell and Molecular Biology Training Grant
1984-present	Participant in the National Cancer Institute Training Grant
1987-present	Participant in National Cancer Institute Program Project "Oncogenes and Development"
1988-present	Participant in Urology Training Grant
1988-present	Participant in Reproductive Biology Training Grant
1991-present	Participant in the Diabetes Center Grant
1992-1999	Participant in P30 Center Core Grant for Reproductive Sciences

Publications
Journal Articles

1. Herr, John C., and J. R. Ellison 1973 Drosophila salivary chromosomes as test system for antinuclear antibody assay. Clin. Exp. Immunol. 15: 451-456.

2. Martin, Lynn, John C Herr, B.A., William Wanamaker, M.D., and Steven Kornguth, Ph.D. 1974 Demonstration of specific antineuronal nuclear antibodies in sera of patients with myasthenia gravis. Indirect and direct immunofluorescence. Neurology 24: 680-683.

3. Herr, John C. 1976 Reflexive gap junctions. Gap junctions between processes arising from the same ovarian decidual cell. J. Cell Biol. 69: 495-501.

4. Larsen, William J., Paul M. Heidger, and John C. Herr 1976 "Central fold" or true junctional profile. J. Cell Biol. 71: 333.

5. Herr, John C. and Paul M. Heidger, Jr. 1977 Decidual cell secretion, a unique mode of exocytosis. Energy Research & Developmental Conference Report-TIC No. 77-504, pp. 88-94.
6. Herr, John C., Paul M. Heidger, Jr., James R. Scott, John W. Anderson, Louis B. Curet, and Harland W. Mossman 1978 Decidual cells in the human ovary at term. 1. Incidence, gross anatomy and ultrastructural features of merocrine secretion. Am. J. Anat. 152: 7-27.

7. Herr, John C., and Paul. M. Heidger, Jr., 1978 A freeze-fracture study of exocytosis and reflexive gap junctions in human ovarian decidual cells. Am. J. Anat. 152: 29-43.

8. Herr, John C., Charles E. Platz, M.D., Paul M. Heidger, Jr., Louis B. Curet, M.D., and C. M. Platz 1979 Smooth muscle within ovarian decidual nodules: a link to leiomyomatosis peritonealis disseminata? Obstet. & Gynec. 53: 451-456.

9. Herr, John C., and E. M. Eddy 1980 Detection of mouse sperm antigens by surface labeling and immunoprecipitation approach. Biol. Reprod. 22: 1263-1274.

10. Allison, Stuart. A., John C. Herr, and J. Michael Schurr 1981 Structure of viral 29 DNA condensed by simple triamines. A light scattering and electron microscopy study. Biopolymers 20: 469-488.

11. Vernon, Robert B., Charles H. Muller, John C. Herr, Frederick A. Feuchter, and E. M. Eddy 1982 Epididymal secretion of a mouse sperm surface component recognized by a monoclonal antibody. Biol. Reprod., 26: 523-535.

12. Eddy, E. M., J. C. Herr, F. A. Feuchter, R. B. Vernon, C. H. Muller, and B. A. Fendersen 1982 The heterogeneity of the sperm surface as analyzed with monoclonal antibodies. Cell Differentiation, 11: 303-304.

13. Herr, John C., Jackson E. Fowler, Stuart S. Howards, Mark Sigman, William M. Sutherland, Deborah J. Koons 1985 Human antisperm monoclonal antibodies constructed postvasectomy. Biology of Reproduction, 32: 695-711.

14. M. Elizabeth H. Bruns, Ellen Kleeman, Stacey E. Mills, David E. Bruns, John C. Herr 1985 The immunochemical localization of vitamin D-dependent calcium-binding protein in mouse placenta and yolk sac. Anat. Rec. 213: 514-517.

15. Herr, John C. and Donald E. Spell 1985 Type A retrovirus particles in heterohybridomas: precaution for human monoclonal antibody purification. J. Ultra. Res. 92: 80-85.

16. Robert J. Evans and John C. Herr 1986 Immunohistochemical localization of the MHS-5 antigen in principal cells of the human seminal vesicle epithelium. Anatomical Record 214: 372-377.

17. Herr, John C., Theresa A. Summers, Robert S. McGee, William M. Sutherland, Mark Sigman, Robert J. Evans 1986 Characterization of a monoclonal antibody to a conserved epitope on human seminal vesicle- specific peptides: A novel probe/marker system for semen identification. Biol. Repro., 35: 773-784.

18. Flickinger, Charles J., John C. Herr and Karin E. Ertl 1986 Identification and isolation of epididymal luminal proteins of the mouse. J. Androl., 7: 163-168.

19. Herr, John C. and Michael P. Woodward 1987 An Enzyme-linked immunosorbent Assay (ELISA) for human semen identification based on a biotinylated monoclonal antibody to a seminal vesicle-specific antigen, J. Forensic Science, 32 (#2): 346-356.

20. Herr, John C., Charles J. Flickinger, Stuart S. Howards, Scott Yarbro, Donald R. Spell, Daniel Caloras and Thomas N. Gallien 1986 An enzyme-linked immunosorbent assay for measuring anti-sperm autoantibodies following vasectomy in Lewis rats. Am. J. Reprod. Immunol. 11: 75-81.

21. Herr, John C., Naugi H. Fares and Mohammed A. Banhawy 1987 Decidual cells in the human ovary at term: II Morphometric analysis of cytoplasmic process and organelles. Am. J. Anat., 179: 277-284.

22. Benjamin, David C., John C. Herr, William M. Sutherland, Michael P. Woodward, Kristi DeCourcey, and Thomas P. Condon 1987 A unique epitope on human serum albumin recognized by monoclonal antibody HSA-1: A probe for identification of the human origin of blood or tissue. Hybridoma, vol. 6, (#2), 183-190.

23. Herr, John C., Charles J. Flickinger, Stuart S. Howards, E. Scott Yarbro, Donald R. Spell, Daniel Caloras and Thomas N. Gallien 1987 Temporal appearance of antisperm autoantibodies in Lewis rats following vasectomy. J. Androl., 8: 253-258.

24. Flickinger, Charles J., Stuart S. Howards, John C. Herr, Daniel Caloras, Thomas N. Gallien, E. Scott Yarbro, and Donald R. Spell 1986 The incidence of spermatic granulomas and their relation to testis weight after vasectomy and vasovasostomy in Lewis rats. J. Andrology 7: 285-291.

25. Kisalus, Lydia L., Wallace C. Nunley, John C. Herr 1987 Protein synthesis and secretion in human decidua of early pregnancy. Biol. Reprod., 36: 785-798.

26. Flickinger, Charles J., John C. Herr, Stuart S. Howards, Daniel Caloras, E. Scott Yarbrough, Donald E. Spell, Thomas N. Gallien 1987 The influence of vasovasostomy on testicular alterations after vasectomy in Lewis rats. Anat. Rec., 217: 137-145.

27. McGee, Robert S. and John C. Herr 1987 Human seminal vesicle-specific antigen during semen liquefaction. Biol. Reprod., 37: 431-439.

28. Herr, John C., Charles J. Flickinger, Stuart S. Howards, Daniel Caloras, E. Scott Yarbro, Donald R. Spell, Thomas N. Gallien 1987 The relation between antisperm antibodies and testicular alterations after vasectomy and vasovasostomy in Lewis rats. Biol. Reprod., 37: 1297-1305.

29. Lydia L. Kisalus, John C. Herr, and Charles D. Little 1987 Immunolocalization of Extracellular matrix proteins and collagen synthesis in first trimester human decidua. Anat. Rec., 218: 402-415.

30. Herr, John C. 1987 Seminal vesicle specific antigen. Clin. Chem. News, 13: 8-9.

31. Flickinger, Charles J., John C. Herr, Kenneth L. Klotz 1988 Immunocytochemical localization of the major glycoprotein of epididymal fluid from the cauda in the epithelium of the mouse epididymis. Cell Tiss. Res. 251: 603-610.

32. Riad, Nahed H., M. Elizabeth Bruns, Nagui H. Fares, David E. Bruns and John C. Herr 1988 Ultrastructural localization of vitamin D-dependent calcium-binding protein in the murine intraplacental yolk sac. Anat. Rec. 222: 252-259.

33. McGee, Robert S. and John C. Herr 1988 Human seminal vesicle-specific antigen is a substrate for prostate specific antigen P30. Biol. Reprod. 39: 499-510.

34. Evans, R. K., Robert S. McGee, T. Summers, Deborah J. Koons, William H. Sutherland, and John C. Herr 1987 Monoclonal antibody: MHS-5 New probe for sexual assault evidence. Virginia Medical 114: 411-415.

35. Carey, Peter O., Stuart S. Howards, Charles J. Flickinger, John C. Herr, Thomas N. Gallien, Daniel Caloras, Donald R. Spell 1988 Effects of granuloma formation at site of vasovasostomy. J. Urol. 139: 853-856.

36. Flickinger, Charles J., Stuart S. Howards, Peter O. Carey, Donald R. Spell, S.J. Kendrick, Daniel Caloras, Thomas N.Gallien and John C. Herr 1988 Testicular alterations are linked to the presence of elevated antisperm antibodies in Sprague-Dawley rats after vasectomy and vasovasostomy. Journal of Urology 140: 627-631.

37. Handley, Harold H., Charles J. Flickinger and John C. Herr 1988 Post - Vasectomy autoantigens in Lewis rat. Biol. Reprod. 39: 1239-1250.

38. Kisalus, Lydia L. and John C. Herr 1988 Immunocytochemical localization of heparan sulfate proteoglycan in human decidual cell secretory bodies and placental fibrinoid. Biol. Reprod. 39: 419-430.

39. Herr, John C., Donald R. Spell, Dorothy J. Conklin, Charles J. Flickinger 1989 Electron microscopic immunolocalization of seminal vesicle - specific antigen in human seminal vesicle. Biol. Reprod. 40: 333-342.

40. Herr, John C., Stuart S. Howards, Donald R. Spell, Peter O. Carey, Sheila J. Kendrick, Thomas N. Gallien Harold Hadley and Charles J. Flickinger 1989 The influence of vasovasostomy on antisperm antibodies in rats. Biol. Reprod. 40: 353-360.

41. Herr, John C., Dorothy Conklin and Robert McGee 1989 Purification of low molecular weight forms of seminal vesicle specific antigen by immunoaffinity chromatography on bound monoclonal antibody MHS-5. J. Reprod. Immunol. 16: 99-113.

42. Herr, John C., Charles J. Flickinger, Mona Homyk, Kenneth Klotz, Edward John 1990 Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. Biol. Reprod. 42: 181-193.

43. Flickinger, C.J., J.C. Herr, S.S. Howards, John R. Sisak, J.M. Gleavy, T.J.Fusia, L.D. Vailes, H. Handley. 1989 Early testicular changes after vasectomy and vasovasostomy in Lewis rats. Anat. Rec. 227: 37-46.

44. Homyk, M., D.J. Anderson, H. Wolff, J.C. Herr 1990 Differential diagnosis of immature germ cells in semen utilizing monoclonal antibody MHS-10 to the intra-acrosomal (protein) SP-10. Fert. Steril. 53: 323-330.

45. Scott, Joanne, Daniel C. Benjamin, John C. Herr, Victor H. Engelhard 1988 Sites of the defects leading to autoimmunity in the spontaneously diabetic BB rat. Adv. Exp. Med. Biol. 246: 53-62.

46. Flickinger, Charles J., John C. Herr, Stuart S. Howards, Daniel Caloras, E. Scott Yarbro, Donald R. Spell, Thomas N. Gallien, John R. Sisak 1990 Inflammatory changes in the epididymis after vasectomy in the Lewis rat. Biol. Reprod. 43: 34-45.

47. Handley, Harold H., Charles J. Flickinger and John C. Herr 1990 Biphasic production of antisperm autoantibodies follows vasectomy of the Lewis rat. J. Reprod. Immunol. 17: 53-67.

48. Herr, John C., Richard M. Wright, Edward John, James Foster, Todd Kays and Charles J. Flickinger 1990 Identification of human acrosomal antigen SP-10 in primates and pigs. Biol. Reprod. 42: 377-382.

49. Coddington, Charles C., David L. Fulgham, Nancy J. Alexander, Deborah Johnson, John C. Herr and Gary D. Hodgen 1990 Sperm bound to zona pellucida in hemizona assay (HZA) demonstrate acrosome reaction when stained with T-6 antibody. Fertil. Steril. 54: 504-508.

50. Wright, Richard M., Edward John, Kenneth Klotz, Charles J. Flickinger and John C. Herr 1990 Cloning and sequencing of cDNAs coding for the human intra-acrosomal antigen SP-10. Biol. Reprod. 42: 693-701.

51. Handley, Harold H., John C. Herr and Charles J. Flickinger 1991 Localization of post-vasectomy sperm autoantigens in the Lewis rat. J. Reprod. Immunol., 20: 205-220.

52. Kurth, Barbara E., Kenneth Klotz, Charles J. Flickinger and John C. Herr 1991 Localization of sperm antigen SP-10 during the six stages of the cycle of the seminiferous epithelium in man. Biol. Reprod. 44: 814-821.

53. Flickinger, Charles J., John C. Herr, Peter O. Carey, E. Scott Yarbro, John R. Sisak, Deborah Williams, Susan Krentz and Stuart S. Howards 1990 Factors that influence fertility after vasovasostomy in Lewis rats. Fertil. Steril. 56: #3, 555-562.

54. Herr, John C., Richard M. Wright, Charles J. Flickinger, Roger L. Eddy and Thomas B. Shows 1991 Assignment of the gene for human intra-acrosomal protein SP-10 to the p12 -> q13 region of chromosome 11. J. Andrology 12: 281-287.

55. Herr, John C., Kenneth Klotz, John Shannon, Richard M. Wright and Charles J. Flickinger 1992 Purification and microsequencing of the intra-acrosomal protein SP-10. Evidence that SP-10 heterogeneity results from endoproteolytic processes. Biol. Reprod. 47: 11-20.

56. Foster, James A. and John C. Herr 1992 Interactions of human sperm acrosomal protein SP-10 with the acrosomal membranes. Biol. Reprod. 46: 981-990.

57. Beecher, Kelly L., Mona Homyk, Chi-Yu Gregory. Lee and John C. Herr 1993 Evidence that 68-Kilodalton and 54-51-Kilodalton Polypeptides are components of the human sperm fibrous sheath. Biol. Reprod. 48: 154-164.

58. Mona Homyk and John C. Herr 1992 Light and electron microscopic immunolocalization of sperm proteins identified by monoclonal antibodies from the World Health Organization taskforce on sperm antigens. J. Reprod. Immunology. 22: 237-256.

59. Flickinger, Charles J., John C. Herr, John R. Sisak, and Stuart S. Howards 1992 ultrastructure of epididymal interstitial reactions after vasectomy and vasovasostomy. Anat. Record 235: 61-73.

60. Freemerman, Alex J., Richard M. Wright, Charles J. Flickinger and John C. Herr 1993 Cloning and sequencing of baboon and cynomolgus monkey intra-acrosomal protein SP-10; homology with human SP-10 and a mouse sperm antigen (MSA-63). Molecular Reproduction and Development 34: 140-148.

61. Shen, M., Richard M. Wright, G. Carta and John C. Herr 1993 Assay for recombinant and native human intra-acrosomal antigen SP-10. Am. J. Reprod. Immunol. 29: 231-240.

62. Fares, Naugi H. and John C. Herr 1993 Computer assisted analysis of electron microscopic images of human decidual cells at term. J. Egypt. Ger. Soc. Zool. 10: 165-184.

63. Kurth, Barbara, Richard M. Wright, Charles J. Flickinger and John C. Herr 1993 Stage-specific detection of mRNA for the sperm antigen SP-10 in human testis. Anat. Record 236: 619-625.

64. Wright, Richard M., Anil K. Suri, Bryan Kornreich, Charles J. Flickinger and John C. Herr 1993 Cloning and characterization of the gene coding for the human acrosomal protein SP-10. Biol. Reproduction 49: 316-325.

65. Golden, Wendy L., Chris von Kap-Herr, Barbara Kurth, Richard M. Wright, Charles J. Flickinger, Roger Eddy, Thomas Shows and John C. Herr 1993 Refinement of the localization of the gene for human intra-acrosomal protein SP-10 (ACRV1) to the junction of bands q23 -> q24 of chromosome 11 by nonisotopic in situ hybridization. Genomics 18, (2): 446-449.

66. Flickinger, Charles J., Mangel Harris, John C. Herr, John Sisak, Stuart S. Howards 1994 Early antibody response following vasectomy is related to fertility after vasovasostomy in glucoconticocil-treated and untreated Lewis rats. J. Urology151: 791-796.

67. Foster, James A., Kenneth Klotz, Charles J. Flickinger, Thomas S. Thomas, Richard M. Wright, James R. Castillo and John C. Herr 1994 Human SP-10. Acrosomal distribution, processing, and fate after the acrosome reaction. Biol. of Reprod.51: 1222-1231.

68. Freemerman, Alex J., Richard M. Wright, Charles J. Flickinger and John C. Herr 1994 Tissue specificity of the Acrosomal Protein SP-10: A contraceptive vaccine candidate molecule. Biol. of Reprod. 50: 615-621.

69. Reddi, P. Prabhakara, James R. Castillo, Kenneth Klotz, Charles J. Flickinger and John C. Herr 1994 Production in Escherichia coli, purification and immunogenicity of acrosomal protein SP-10, a candidate contraceptive vaccine. Gene 147: 189-195.

70. Flickinger, Charles J., Stuart S. Howards, Leigh Ann Bush. Linda A. Baher and John C. Herr 1995 Antisperm autoantibody responses to vasectomy and vasovasostomy in Lewis and Fisher rats. J. Reprod. Immunology 28: 137-157.

71. Flickinger, Charles J., Stuart S. Howards, Leigh Ann Bush, Linda A. Baher and John C. Herr 1994 Temporal recognition of sperm autoantigens by IgM and IgG autoantibodies after vasectomy and vasovasostomy. J. Reprod. Immunol. 27: 135-150.

72. Fusi, Francesco M., I. Lorenzetli, F. Mangili, John C. Herr, Alex J. Freemerman, James Gailit and Richard Bronson 1994 Vitronectin is an intrinsic protein of human spermatozoa released during the acrosomal reaction. Mol. Reprod. Dev. 39: 337-343.

73. Jeung, E-B, Nancy C. Fan, Peter C.K. Leung, John C. Herr, Alex Freemerman and John Krisinger 1995 The baboon expresses the calbindin-9 kd gene in intestine but not in uterus and placenta: Implications for conservation of the gene in primates, Mol. Reprod. & Dev. 40: 400-407.

74. Dandulakis, Gregory, John C. Herr and Donald J. Kirwan 1994 Physiological responses of hybridoma cells in a protein-free medium to soluble and immobilized antigen. Biotechnology and Bioengineering 44: 1155-1159.

75. Flickinger, Charles J., John C. Herr, Maureen Baran, and Stuart Howards 1995 Testicular development and the formation of spermatic granulomas of the epididymis after obstruction of the vas deferens in immature rats. J. Urology 154: 1539-1544.

76. Flickinger, Charles J., John C. Herr, Maureen Baran and Stuart Howards 1995 Temporal appearance of antisperm antibodies during sexual maturation of rats with obstruction of the vas deferens. J. Andrology 16: 75-79.

77. Srinivasan, J., S. Tinge, Richard Wright, John C. Herr, Ron Curtiss III 1995 Oral immunization with attenuated Salmonella expressing human sperm antigen induces antibodies in serum and the reproductive tract. Biol. Reproduction 53: 462-471.

78. Freemerman, Alex J., Charles J. Flickinger and John C. Herr 1995 Characterization of alternatively spliced human SP-10 mRNAs. Mol. Reprod. Dev. 41: 100-108.

79. Dandulakis, Gregory, John C. Herr, Donald J. Kirwan 1995 Cell growth and monoclonal antibody production in the presence of antigen and serum. Biotechnology Progress 11: 518-524.

80. Tatsuhiro Yoshiki, John C. Herr and Chi-Yu Gregory Lee 1995 Purification and characterization of a sperm antigen recognized by HSA-5 monoclonal antibody. J. Reprod. Immunol. 29: 209-222.

81. Chu-Yu Gregory Lee, Tatsuhiro Yoshiki, Leo Chui, Patricia McChesney, John C. Herr, Eung-Soo Hwang and Eng-Shang Huang 1995 Epitope analysis of a sperm acrosomal antigen defined by HSA-5 monoclonal antibody. J. Reprod. Immunol. 29: 223-238.

82. Reddi, P. Prabhakara, Soren Naaby-Hansen, Irena Aguolnik, Jen-Yue Tsia, Lee M. Silver, Charles J. Flickinger and John C. Herr 1995 Complementary Deoxyibonucleic Acid Cloning and Characterization of mSP-10: The Mouse Homologue of Human Acrosomal Protein SP-10. Biology of Reproduction 53: 873-881.

83. S. A. Coonrod, J. C. Herr and M.E. Westhusin 1996 Inhibition of bovine fertilization in vitro by antibodies to SP-10. J. Reprod. Fertility 107: 287-297.

84. Charles Flickinger, Maureen Baran, Stuart S. Howards and John C. Herr 1996 Sperm autoantigens recognized by autoantibodies in developing rats following prepuberal obstruction of the vas deferens. J. Androl. 17: 433-442.

85. Michael J. Wolkowicz, Scott A. Coonrod and John C. Herr 1996 Refinement of the differentiated phenotype of the spermatogenic cell line GC-2spd (ts). Biol. Reprod. 55: 923-932.

86. Charles Flickinger, Stuart S. Howards, Maureen Baran, Nikki Pessoa and John C. Herr 1997 Appearance of natural anti-sperm autoantibodies after sexual maturation of normal Lewis rats. J. Reprod. Immunol. 33 (2): 127-145.

87. Cecelia Videaus, Chris Von Kap-Herr, Wendy Golden. Roger L. Eddy, Thomas B. Shows and John C. Herr 1997 Human fertilin: Identification characterization and chromosomal mapping of an ADAM gene family member. Mol. Reprod. Dev. 46: 363-369.

88. Soren Naaby-Hansen, Charles J. Flickinger and John C. Herr 1997 Two-dimensional gel electrophoretic analysis of vectorially labeled human spermatozoa surface proteins. Biol. Reprod. 56: 771-787.

89. Alan B. Diekman and John C. Herr 1997 Sperm antigens and their use in the development of an immunocontraceptive. Am. J. Reprod. Immunol. 37: 111-117.

90. Sivashanmugam, P., John C. Herr, and M. Rajalakshmi 1996 Immunofluorescence localization of SP 10 protein on monkey spermatozoa during maturation. Current Science 70 (12): 1075-1076.

91. Charles J. Flickinger, Leigh Ann Bush, Stuart S. Howards, and John C. Herr 1997 Distribution of leukocytes in the epithelium and interstitium of four regions of the Lewis rat epididymis. Anat. Rec. 248: 380-390.

92. Soren Naaby-Hansen, Richard W. Roof, Charles J. Flickinger, Constance M. Grafer, Sarah J. Parsons, and John C. Herr 1997 A novel negative imaging technique for accurate localization of stainable proteins on complex (Two-dimensional) autoradiograms. Electrophoresis 18: 2065-2070.

93. Barbara E. Kurth, Clifton Weston, P. Prabhakara Reddi, Donald Bryant, Renuka Bhattacharya, Charles J. Flickinger, and John C. Herr 1997 Oviductal antibody response to a defined recombinant sperm antigen in macaques. Biol. Reprod. 57: 981-989.

94. Dandulakis, Gregory., John C. Herr and Donald J. Kirwan 1997 Effect of growth factors and antigen on hybridoma cell culture dynamics. Biotechnology and Bioengineering, 54 (4): 357-364.

95. Diekman, Alan B., V. Anne Westbrook-Case, Soren Naaby-Hansen, Kenneth L. Klotz, Charles J. Flickinger and John C. Herr 1997 Biochemical characterization of sperm aggutination antigen-1, a human sperm surface antigen implicated in gamete interactions. Biol. Reprod. 57: 1136-1144.

96. Flickinger, Charles J., Maureen L. Baran, Stuart S. Howards and John C. Herr 1998 Epididymal obstruction during development results in antisperm autoantibodies at puberty in rats. J. Androl. 19: 136-144.

97. Kurth, Barbara E., Donald Bryant, Soren Naaby-Hansen, P. Prabhakara Reddi, Clifton Weston, Patricia Foley, Renuka Bhattacharya, Charles J. Flickinger and John C. Herr 1997 Immunological response in the primate oviduct to a defined recombinant sperm immunogen. J. of Reprod. Immunol. 35: 135-150.

98. Flickinger, Charles J, Maureen L. Baran, Stuart S. Howards and John C. Herr 1999 Degeneration of the seminiferous epithelium following epididymal obstruction in prepubertal rats. Anat. Rec 254: 76-86.

99. P. Prabhakara Reddi, Marko Kallio, and John C. Herr 1999 Green fluorescent protein as a reporter for promoter analysis of testis-specific genes in transgenic mice. Methods in Enzymology 302: 272-284.

100. Scott A. Coonrod, Soren-Naaby-Hansen, Jagathpala Shetty, Hiro Shibahara, Michellee Chen,

Judith M. White, and John C. Herr 1999 Treatment of mouse oocytes with PI-PLC releases 70 kDa (pI 5) and 35-45 kDa (pI5.5) protein clusters from the egg surface and inhibits sperm-oolemma binding and fusion. Developmental Biol. 207: 334-349

101. Alan B. Diekman, Elizabeth J. Norton, Kenneth L. Klotz, V. Anne Westbrook, Hiroaki Shibahara, Soren Naaby-Hansen, Charles J. Flickinger, and John C. Herr 1999 N-linked glycan of a sperm CD52 glycoform associated with human infertility. FASEB Journal 13: 1303-1313.

102. Jagathpala Shetty, Soren Naaby-Hansen, Hiroaki Shibahara, Richard Bronson, Charles Flickinger, and John C. Herr 1999 Human Sperm Proteome Immunodominant sperm antigens identified with sera from infertile men and women. Biol. Reproduction 61: 61-69.

103. Charles J. Flickinger, Leigh Ann Bush, Mollie V. Williams, Soren Naaby-Hansen, Stuart S. Howards, and John C. Herr 1999 Post-obstruction rat sperm autoantigens identified by two-dimensional gel electrophoresis and western blotting. J. Reprod. Immunol. 43: 35-53.

104. John C. Herr, David Thomas, Leigh Ann Bush, Scott Coonrod, Vrinda Khole, Stuart S. Howards and Charles J. Flickinger 1999 Sperm mitochondria- associated cysteine-rich protein [SMCP] is autoantigen in Lewis rats. Biol. Reprod. 61: 428-435.

105. Scott Coonrod, Soren Naaby-Hansen, Jagathpala Shetty, and John C. Herr 1999 PI-PLC releases a 25-40 kDa protein cluster from the hamster oolemma and affects the sperm penetration assay. Molecular Human Reproduction Vol. 5 No.11: 1027-1033.

106. Alan B. Diekman, Elizabeth J. Norton, Kenneth L. Klotz, V. Anne Westbrook, John C. Herr 1999 Evidence for a unique N-linked glycan associated with human infertility on sperm CD52: A candidate contraceptive vaccinogen. Immunological Reviews: 171: 203-212.

107. Arabinda Mandal, Soren Naaby-Hansen, Michael J. Wolkowicz, Ken Klotz, Jagathpala Shetty, Jacques D. Retief, Scott A. Coonrod, Michael Kinter, Nicholas Sherman, Fred Cesar, Charles J. Flickinger and John C. Herr 1999 FSP95, a testis specific, 95-Kilodalton fibrous sheath antigen, that undergoes tyrosine phosphorylation in capacitated human spermatozoa. Biology of Reproduction 61: 1184-1197.

108. P. Prabhakara Reddi, Charles J. Flickinger and John C. Herr 1999 Round spermatid-specific transcription of the mouse SP-10 gene is mediated by a 294-base pair proximal promoter. Biology of Reproduction 61: 1256-1266.

109. Michellee S. Chen, Kenneth S. K. Tung, Scott A. Coonrod, Yuji Takahashi, Dora Bigler, Alice Chang, Y. Yamashita, P.W. Kincade, John C. Herr and Judith M. White 1999 Role of the intergrin-associated protein, CD9, in binding in between sperm ADAM 2 and the egg intergrin 6 1: implications for murine fertilization. P.N.A.S. 96: 11830-11835.

110. Charles J. Flickinger, Mark Vagnetti, Stuart S. Howards and John C. Herr 2000 Antisperm autoantibody response is reduced by early repair of a severed vas deferens in the juvenile rat. Fertility and Sterility 73: 229-237.

111. John C. Herr, Kenneth L. Klotz, Peggy Anderson, Edward Adams, Norman Moore, and Stuart Howards 1999 Progress in developing an immunochromatographic device for sperm detection. Clinical Immunology Newsletter Vol 19 (4/5): 52-58.

112. Diekman, Alan B., Elizabeth J. Norton, V. Anne Westbrook, Kenneth L. Klotz, oren Naaby-Hansen and John C. Herr 2000 Anti-sperm antibodies from infertile patients and their cognate sperm antigens: (A review) identity between SAGA-1,the H6-3C4 antigen, and CD52. Am. J. of Reprod. Immunol. 43: 134-143.

113. V. Anne Westbrook, Alan B. Diekman, Kenneth L. Klotz, Vrinda V. Khole, Chris von Kap-Herr, Wendy L. Golden, Roger L. Eddy, Thomas B. Shows, Mark H. Stoler, Charles J. Flickinger and John C. Herr. 2000 Spermatid-specific expression of the novel X-linked gene product SPAN-X localized to the nucleus of human spermatozoa. Biol. Reprod. 63: 469-481.

114. V. Anne Westbrook, Alan B. Diekman, Soren Naaby-Hansen, Scott A. Coonrod, Kenneth Klotz, Theodore S. Thomas, Elizabeth J. Norton, Charles J. Flickinger, John C. Herr. 2001 Localization of the SPAN-X protein, a cancer/testis marker, to nuclear vaculoles and redundant nuclear envelope of human spermatozoa. Biol. Reprod. 64: 345-358.

115. E. J. Norton, A. B. Diekman, V. A. Westbrook, C. J. Flickinger and J. C. Herr, 2001 RASA: An anti-sperm ScFv directed against the human sperm surface: Implications for novel contraceptives. Human Reproduction 16. (#9) 1854-1860.

116. Regina M. O. Turner, Mariana P. Musse, Arabinda Mandal, Ken Klotz, Friederike Jayes and John C. Herr, George L. Gerton, Stuart B. Moss, and Hector E. Chemes. 2000 Molecular geneti analysis of two human sperm fibrous sheath proteins, AKAP4 and AKAP3, in men with dysplasia of the fibrous sheath. J. Androl. 22: 302-315.

117. Charles J. Flickinger, Jayasimha Rao, Leigh Ann Bush, Nicholas E. Sherman, Richard J. Oko, Friederike C.L. Jayes and John C. Herr. 2001 Outer dense fiber proteins are dominant postobstruction autoantigens in adult Lewis rats. Biol. Reprod. 64: 1451-1459.

118. Jagathpala Shetty, Alan Diekman, Friederike Jayes, Nicolas Sherman, Soren Naaby-Hansen and John C. Herr. 2001 Differential extraction and enrichment of human sperm surface proteins in a 2-D proteome: Identification of immunocontraceptive candidates. Electrophoresis 22: 3053-3066.

119. Soren Naaby-Hansen, Michael J. Wolkowicz, Ken Klotz, Leigh Ann Bush, V. Anne Westbrook, Hiroaki Shibahara, Jagathpala Shetty, Scott A. Coonrod, P. Prabhakara Reddi, John Shannon*, Michael Kinter*, Nicholas Sherman* Jay Fox*, Charles J. Flickinger and John C. Herr. 2001 Co-localization of the inositol 1,4,5-trisphosphate receptor and calreticulin in the equatorial segment and in membrane bounded vesicles in the cytoplasmic droplet of human spermatozoa. Mol. Human Reproduction 7 (10) 923-933.

120. Scott A. Coonrod, Paul W. Wright, and John C. Herr. 2001 Oolemmal; Proteomics. J. Reprod. Immunol. 53: 55-65.

121. Leigh Ann Bush, John C. Herr, Michael Wolkowicz, Nicholas E. Sherman, Amy Shore and Charles J. Flickinger. 2001 A Novel Asparaginase-like protein is a sperm autoantigen in rats. Mol. Reprod. Dev. 62 (2) 233-247.

122. P. Prabhakara Reddi, Amy N. Shore, Kshitish K. Acharya and John C. Herr 2001 Transcriptional Regulation of Spermiogenosis: Insights from the Study of the Gene encoding the Acrosomal Protein SP-10. J. Reprod. Immunol. 53: 25-36.

123. Scott A. Coonrod, Laura C. Bolling, Paul W. Wright, Pablo E. Visconti and John C. Herr 2001 A Morpholino Phenocopy of the Mouse Mos Mutation. Genesis 30: 198-200.

124. Michael J. Wolkowicz, Soren Naaby-Hansen, Angela R. Gamble, P. Prabhakara Reddi, Charles J. Flickinger and John C. Herr. 2002 Tektin B1 Demonstrates Flagellar Localization in Human Sperm. Biol. of Reprod. 66: 241-250.

125. Pablo E. Visconti, Zhonglin Hao, Marie Purdon, Paula Stein, Binaifer R. Balsara, Joseph R. Testa, John C. Herr, Stuart Moss, and Gregory S. Kopf. 2001 Cloning and chromosomal localization of a novel serine/threonine kinase belonging to the subfamily of testis-specific kinases. Genomics 77: 163-170.

126. Tod C. McCauley, Barbara E. Kurth, Elizabeth J. Norton, Kenneth L. Klotz, V. Anne Westbrook, A. Jaganandha Rao, John C. Herr, and Alan B. Diekman. 2002 Analysis of a Human Sperm CD52 Glycoform in Primates: identification of an animal model for immunocontraceptive vaccine development. Biol. Of Reprod.66: 1681-1688.

127. Zhonglin Hao, Michael J. Wolkowicz, Jagathpala Shetty, Kenneth Klotz, Laura Bolling, Sen Buer, V. Anne Westbrook, Scott Coonrod, Charles J. Flickinger and John C. Herr. 2002 SAMP32, a testis-specific, isoantigenic acrosomal membrane-associated protein. Biol. of Reprod. 66: 735-744.

128. Soren Naaby-Hansen, Arabinda Mandal, Michael J. Wolkowicz, Sen Buer, Jacque Retief, V. Anne Westbrook, Jagathpala Shetty, Michael Cox, Friederike Jayes, Scott A. Coonrod, Kenneth L. Klotz, Leigh Ann Bush, Charles J. Flickinger, and John C. Herr. 2002 CABYR, a novel calcium binding tyrosine phosphorylation regulated fibrous sheath protein involved in capacitation. Dev. Biol. 242: 236-254.

129. Hiroaki Shibahara, Ikuo Sato, Jagathpala Shetty, Soren Naaby-Hansen, John C. Herr, Eiko Wakimoto and Koji Koyama. 2002 Two-dimensional electrophoretic analysis of sperm antigens recognized by sperm immobilizing antibodies detected in infertile women. J. Reprod. Immunol. 53: 1-12.

130. Zhonglin Hao, Anil Suri, Sen Buer, Amy Shore, V. Anne Westbrook, Charles J. Flickinger, John C. Herr, and Scott A. Coonrod. 2002 TACC3 Expression and Localization in the Murine Egg and Ovary. Mol. Reprod. Dev. 63:291-299.

131. Jagathpala Shetty, Michael J. Wolkowicz, Kenneth L. Klotz, Friederike L. Jayes, Alan Diekman, Laura Bolling, V. Anne Westbrook, Erin M. Dickinson, Scott Coonrod, Charles J. Flickinger,

and John C. Herr. 2003 SAMP14, a Novel, Acrosomal Membrane Associated, GPI anchored member of the Ly-6/UPAR Receptor Superfamily with a Role in Sperm-Egg Interaction. J. Biol. of Chem. Vol. 278: 30506-30515

132. Jayasimha Rao, John C. Herr, Prabhakara Reddi, Michael J. Wolkowicz, Leigh Ann Bush, Nicholas E. Sherman, Michael Black, and Charles J. Flickinger. 2003 Cloning and -characterization of a novel sperm associated iso-antigen (E-3) with defensin/lectin-like motifs expressed in rat epididymidis. Biol. of Reprod. 68: 290 – 301.

133. Li-Xin Feng, Yali Chen, Luis Dettin, Renee A. Reijo Pera, John C. Herr, Erwin Goldberg and Martin Dym. 2002 Generation and In Vitro Differentiation of a Spermatogonial Cell Line. Science 297: 392-395.

134. Buer, Sen, Arabinda Mandal, Michael J. Wolkowicz, Prabhakara Reddi, Jagathpala Shetty, Leigh Ann Bush, Charles J. Flickinger and John C. Herr. 2003 Cloning and Characterization of the Murine Calcium Binding, Tyrosine Phosphorylated Fibrous Sheath Protein, CABYR. Gene 310:67-78.

135. Elizabeth J. Norton, Alan B. Diekman, V. Anne Westbrook, Kenneth L. Klotz, Theodore S. Thomas, D. Craig Wright, Joan Brisker, Charles J. Flickinger, D.W. Mullins, L.L. Gilmer, V.H. Engelhard and John C. Herr 2002 A unique, male genital tract-specific, carbohydrate epitope on human CD52: Implications for immunocontraception. Tissue Antigens 60: 354-364

136. Arabinda Mandal, Kenneth L. Klotz, Jagathpala Shetty, Friederike L. Jayes, Michael J. Wolkowicz, Laura C. Bolling, Scott A. Coonrod, Michael B. Black, Alan B. Diekman, Timothy A.J. Haystead, Charles J. Flickinger and John C. Herr. 2003 SLLP1, A Unique, Intra-Acrosomal, Non-Bacteriolytic, c Lysozyme-Like Protein in Human Spermatozoa. Biol. of Reprod. 68:1525-1537.

137. Wolkowicz, M.J., Shetty, J., Westbrook, A., Bolling, L., Klotz, K., Coonrod, S., Jayes, F., Mandal, A., Flickinger, C.J., and Herr, J.C. 2003 Equatorial Segment Protein (ESP): An Alloantigenic Glycoprotein that Mediates Sperm-Egg Interactions and Provides Evidence for an Equatorial Segment Subcompartment in Acrosomal Vesicle Stage Spermatids. Biol. Reprod. 69: 735-745.

138. M.J. Wolkowicz, J. Shetty, L. Bolling, S. Conrood, K. Klotz, C.J. Flickinger, and J.C. Herr 2003 Equatorial Segment Protein (ESP) is a Human Alloantigen Involved in Sperm-egg Binding and Fusion. Mol. Human Reprod. (Submitted).

139. Meredith E. Calvert, Laura C. Bolling, John C. Herr, and Scott A. Coonrod, 2003 Oolemmal Proteomics: Identification of highly abundant heat shock proteins and molecular chaperones in the mature mammalian egg and their localization on the plasma membrane. Reprod. Biol. Endocrinology 1:27 (14 Feb 2003).

140. Paul W. Wright, Laura C. Bolling, Meredith E. Calvert, Olga F. Sarmento, Elizabeth V. Berkeley, Margaret C. Shea, Zhonglin Hao, Friederike C. Jayes, Leigh Ann Bush, Jagathpala Shetty, Amy N. Shore, Prabhakara P. Reddi, Kenneth S. Tung, Eileen Samy, Margaretta M. Allietta, Nicholas E. Sherman, John C. Herr, and Scott A. Coonrod. 2003 ePAD, an Oocyte and Early

Embryo-Abundant Peptidylarginine Deiminase-Like Protein which Localizes to Egg Cytoplasmic Sheets. Dev. Biol. 256:73-88.

141. Scott Ficarro, Olga Chertihin, V. Anne Westbrook, Forest White, Friederike Jayes, Petr Kalab, Jarrod A. Marto, Jeffrey Shabanowitz, John C. Herr, Don Hunt, and Pablo E. Visconti. 2003 Phosphorproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of AKAP 3 and valosin containing protein/p97 during capacitation. J. Biol. Chem. 278:11579-11589.

142. S.A. Coonrod, M.E. Calvert, P.P. Reddi, E.N. Kasper, L.C. Digilio, and J.C. Herr. 2004, Oocyte proteomics: localisation of mouse zona pellucida protein 3 to the plasma membrane of ovulated mouse eggs. Reprod. Fertility and Development.16, 1-10.

143. Zhonglin Hao, Kula N. Jha, Young-Hwan Kim, Soumya Vemuganti, V. Anne Westbrook, Olga Chertihin, Karin Markgraf, LaRhonda Jackson, Charles J. Flickinger, Michael Coppola, John C. Herr, and Pablo E. Visconti. 2004 Expression analysis of the human testis specific ser/thr Kinases (TSSK) homologues. A TSSK member is present in the equatorial segment of human sperm. Mol. Human Reprod. 10, #6, 433-444.

144. V.A. Westbrook, P.D. Schoppee, A.B. Diekman, K.L. Klotz, M. Allietta, K. Hogan, C. Slingluff, J. Patterson, H. Frierson, W.P. Irvin Jr., C.J. Flickinger, M.A. Coppola, J.C. Herr, 2004. Genomic Organization, Incidence and Localization of the SPAN-X Family of Cancer-Testis Antigens in Melanoma Tumors and Cell Lines. Clin. Cancer Res. 10:101-112.

145. Zhiqing Wang, Yana Zhang, Arabinda Mandal, Jian Zhang, Francis J. Giles, John C. Herr, and Seah H. Lim. 2004 The spermatozoa protein, SLLP1, is a novel Cancer-Testis antigen in hematologic malignancies. Clin. Cancer Research 10(19):6544-50.

146. Maria Belen Herrero, Arabinda Mandal, Laura C. Digilio, Scott Coonrod, Bernhard Maier, John C. Herr. 2005 A unique c-lysosome-like protein has a role in fertilization. Developmental Biology 284: 126-142.

147. Olga F. Sarmento, Laura C. Digilio, Yanming Wang, Julie Perlin, John C. Herr, C. David Allis and Scott A. Coonrod. 2004 Dynamic Alterations of Specific Histone Modifications During Early Murine Development. Journal of Cell Science. 117(Pt 19):4449-59.

148. Susan Sleight, Patricia Miranda, Nia-Washington Plaskett, Bernhard Maier, Jeff Lysiak, Heide Scrable, John C. Herr and Pablo E. Visconti. 2005 Disruption of Mouse Sperm Lipid Rafts During Capacitation and Analysis of Raft Domain Proteins" Biology of Reproduction 73(4): 721-9 .

149. Jayasimha Rao, John C. Herr, P. Prabhakara Reddi, Mark H. Stoler, Amy Shore, Dirk R. Yonkee, Leigh Ann Bush and Charles J. Flickinger. 2005 Region-Specific Expression of the Sperm Associated Defensin-and Lectin-Like Epididymal Protein E3. Biology of Reproduction (submitted)

150. V. Anne Westbrook, Pamela D. Schoppee, Geeta R. Vanage, Ken L. Klotz, Alan B. Diekman, Charles J. Flickinger, Michael A. Coppola, John C. Herr 2005 Spermatid Nuclear Envelope Morphogenesis Evidenced by Localization of the SPAN-X Cancer/Testis Antigen During Human and Chimpanzee Spermiogenesis. The New Anatomist [in press]

151. Nirmala Jagadish, Ritu Rana, Ramasamy Selvi, Deepshikha Mishra, Manog Garg, Shikha Yadav, John C. Herr, Katsuzumi Okumura, Akiko Hasegawa, Koji Koyama, Anil Suri. 2005 Characterization of a novel human sperm associated antigen 9 (SPAG9) having structural homology with α-Jun NH2-terminal kinase interacting protein. Biochemical Journal 389(Pt 1):73-82.

152. Clara I. Marin-Briggiler, Kula Nand Jha, Olga Chertihin, Mariano G. Buffone, John C. Herr, Moniza H. Vazquez-Levin and Pablo Visconti. 2005 Evidence of the Presence of Calcium/Calmodulin-Dependent Protein Kinase IV in Human Sperm and its Involvement in Motility Regulation J. Cell Science 118 (9): 2013-2022..

153. Young-Hwan Kim, Kula N. Jha, Arabinda Mandal, Geeta Vanage, Erin Farris, Phillip L. Snow, Ken Klotz, Charles J. Flickinger and John C. Herr 2005 Translation and Assembly of CABYR Coding Region B in Fibrous Sheath and Restriction of Calcium Binding to Coding Region A. Dev. Biol. 286: 46-56.

154. Vitale A. Perlin J. Leonelli L. Herr J. Wright P. Digilio L. Coonrod S. Mouse cPLA2gamma, a novel oocyte and early embryo-abundant phospholipase A2 gamma-like protein, is targeted to the nuclear envelope during germinal vesicle breakdown. 2005 Developmental Biology. 282(2):374-84.

155. Scott Coonrod, Alejandra Vitale, Chongwen Duan, John Herr, Sarah Bristol-Gould and Erwin Goldberg 2006 Expression of Testis-Specific Lacatate Dehydrogenase (LDH-C_4; Ldh3) in Murine Oocytes and Preimplantation Embryos. J. Androl. [in press].

156. B. Xu, Z. Hao, K. N. Jha, L. Digilio, C. Urekar, YH. Kim, W. He, S. Pulido, C. J. Flickinger, J. C. Herr 2006 Validation of a testis specific serine/threonine kinase [TSSK] family, and the substrate of TSSK1 & 2, TSKS, as contraceptive targets. Reproduction [in press]

157. Bingfang Xu, Michael Copolla, John C. Herr, and Michael P. Timko 2006 Expression of a recombinant human sperm-agglutinating miniantibody in tobacco (*Nicotiana tabacum* L.). Reproduction [in press]

Book Chapters

1. Benjamin, D. C., J. C. Herr, M. P. Woodward 1986 Immunologic basis of species differentiation. Proceedings of the International Symposium on Forensic Immunol., Lib. Congress #87-619829, pub. by U.S. Dept. Justice, p. 15-22.

2. Herr, J. C., D. C. Benjamin, M. J. Woodward 1988 Detection of the human origin of blood or tissue using a monoclonal antibody specific to human albumin. Advances in Chemistry Series 220, Archaeological Chemistry IV, R.O. Allen, ed., pub. by Am. Chem. Soc., p. 389-406.

3. Herr, J.C., R.M Wright, C.J. Flickinger. 1990 Biochemical, ultrastructural and genetic characterization of human acrosomal antigen SP-10. Proceedings of the 4th International Congress of Reproductive Immunology. L. Mettler, WD Billington, eds. Elsevier, Amsterdam p. 51-59.

4. Herr, J.C., R.M. Wright C.J. Flickinger 1990 Monoclonal antibody MHS-10 and its cognate intra-acrosomal immunogen, SP-10. In : "Gamete Interaction; Prospects for Immunocontraception" Ed: NJ. Alexander, D. Griffin, J.M. Spieler, G M.H. Waites, Wiley-Liss p. 13-36.

5. Herr, J.C. 1992 Organization and direction of the Center for Recombinant Gamete Contraceptive Vaccinogens. In "Opportunities in Contraception: Research and Development" ed, F. Hazeltine, American Association for the Advancement of Science Press.

6. Herr, J.C., R.M. Wright, C.J. Flickinger, A. Freemerman, K. Klotz, J. Castillo 1997 Update on sperm antigen SP-10. In: Immunological Aspects of Reproductive Health, eds., P.D. Griffin and G.T. Sukhikh. Proceedings of World Health Organization 20th Anniversary Symposium held in Moscow Russian Federation, p. 27-40.

7. Herr, J.C., R.M. Wright, C.J. Flickinger, A. Freemerman, K. Klotz, J. Foster, J. Shannon 1993 Intra-acrosomal contraceptive vaccine immunogen SP-10 in man, macaque and baboon. In:" In Vitro Fertilization and Embryo Transfer in Primates". R.M. Brenner, D.P. Wolf, R.L. Stouffer, eds. Springer-Verlag, N.Y., p. 360-383.

8. Wright, R.M., A.K. Suri, J.C. Herr 1993 Locus and structure of the gene encoding the human intra-acrosomal protein SP-10. In: "Reproductive Immunology", F. Dondero and P.M. Johnson, eds., Serona Symposia, Raven Press, New York, Vol. 97, p. 47-55

9. Herr, J.C., R.M. Wright, K. Klotz, M. Homyk, B. Kornreich, C.J. Flickinger, J. Powell, V. Stevens 1993 Immunogenicity of SP-10 fusion proteins in female baboons. In: "Symposium on Recombinant and Synthetic Vaccines," New Delhi, India, December 1992, Narosa Publishing House, Delhi, India, p. 239-251.

10. Curtiss III, Roy, J. O. Hassan, J.C. Herr, S.M. Kelly, M. Levine, G.G. Mahairas, D. Milich, D. Peterson, F. Schodel, J. Srinivasan, C. Tacket, S.A. Tinge, R.M. Wright 1994 Nonrecombinant and recombinant avirulent Salmonella vaccines. In: "Symposium on Recombinant and Synthetic Vaccines," New Delhi, India, December 1992, Narosa Publishing House, Delhi, India, p. 340-351.

11. Herr, J.C., C.J. Flickinger, S.S. Howards 1994 The immunobiological effects of vasectomy and vasovasostomy in the rat model. In: Immunobiology of Reproduction, Joan S. Hunt, ed., Springer-Verlag, p. 254-284.

12. Flickinger, C.J., S.S. Howards, J.C. Herr 1995 Effects of vasectomy on the epididymis. J. of Microscopy Research and Techniques 30: 82-100.

13. Herr, J.C. 1994 Antifertility vaccines - The promising candidates. In: "Fertility Control", second edition, Steven L. Corson, Richard J. Derman, Louise B. Tyrer, eds., Goldin Publishers, London, Ont., Canada. p. 431-452.

14. Herr, J.C., A.B. Diekman, S.A.Coonrod, A.J. Freemerman 1995 Recent advances on the path to a contraceptive vaccine. In: "Fertility and Sterility, A Current Overview, Parthenon, London, p. 35-46.

15. Herr, J.C. 1995 The use of acrosomal antigens as contraceptive immunogens: studies on the intra-acrosomal antigen SP-10. In: "Human Sperm Acrosome Reaction", John Libby Eurotext, LTD. INSERM, p. 373-384.

16. Herr, J.C. 1996 Update on the Center for Recombinant Gamete Contraceptive Vaccinogens. Proceedings of the Sixth International Congress of Reproductive Immunology, Am. J. Reprod. Immunol. 35:184-189.

17. Herr, J.C. 1996 Immunocontraceptive approaches In: "Contraceptive Research and Development", National Academy Press, eds, Polly F. Harrison and Allan Rosenfield, p. 401-429.

18. Erwin Goldberg and John C. Herr 1999 LDH-C_4 as a contraceptive vaccine. In: "Reproductive Immunology", S.K. Gupta, ed. Narosa Pub. House, New Delhi, India. p 309-315.

19. John C. Herr 2002 Update on Sperm Antigen Based Contraceptive Vaccines. In: Immunology and Allergy Clinics of North America, 22:503-527. A. Arici, ed. Hartcourt, Philadelphia, PA

20. A.B. Diekman and J.C. Herr 2003 Overview of human sperm surface antigens acquired during epididymal maturation. In: The Third International Conference on the Epididymis, The van Doren Company, Charlottesville, VA. p184-191. eds, Barry Hinton and Terrry Turner.

Invited Articles

1. Flickinger, C.J., J.C. Herr, R.S. McGee, R.J. Evans, W.M. Sutherland, M. Sigman, T.A. Summers, D.R. Spell, D.J. Conklin 1990 Dynamics of a human seminal vesicle specific protein. Proceedings International Workshop on Seminal and Sperm Specific Proteins in Honor of T. Mann, September, 1989. Andrologia 22, Suppl 1, 142-154.

2. Freemerman, A.J., R.M. Wright, C.J. Flickinger, J.C. Herr 1994 Update on the tissue specificity of the acrosomal protein SP-10: A contraceptive vaccine candidate molecule. Proceedings of the 8th Annual Meeting of the Japanese Society for the Immunology of Reproduction. Y. Tsunoda, ed. p. 1-8.

Patents Developed at Virginia and Held by UVA Patent Foundation (updated 2/05)

A. Issued U.S. Patents

1. Inventors: J.C. Herr, M. Sigman and W. Sutherland
   Title: "Monoclonal Antibody to MHS-5: a New Probe for Sexual Assault Analyses"
   UVAPF Docket no. 0272-01 (Herr-MHS-5)

Filed June 5, 1985
U.S. Patent 4,741,998 Issued May 3, 1988

2. Inventors: J C. Herr, M. Sigman,and W. Sutherland
Title: "Monoclonal Antibody to MHS-5; a New Probe for Sexual Assault Analysis"
UVAPF Docket no. 0272-02 (Herr-MHS-5)
Filed April 21, 1987
U.S. Patent 5,047,508 Issued Sept. 10, 1991

3. Inventors: J.C. Herr and D. Benjamin
Title: "Monoclonal Antibodies and Method of Identifying Species Using the Same"
UVAPF Docket no. 0278-01 (Herr-HSA-1)
Filed July 16, 1985
U.S. Patent 4,735,898 Issued April 5, 1988

4. Inventors: J.C. Herr and R. M. Wright
Title: "Human Intra-Acrosomal Sperm Antigen"
UVAPF Docket no. 0215-02 (Herr-Sprmagp)
Filed on Feb. 16, 1990
US Patent 5,436,157 Issued July 25, 1995

5. Inventors: J.C. Herr and R.M. Wright
Title: "Primate Intra-Acrosomal Sperm Antigen for Use in a Contraceptive Vaccine"
UVAPF Docket no. 0215-04 (Herr-Sprmagp)
Filed Aug. 18, 1984
U.S. Patent 5,602,005 Issued Feb. 11, 1997

6. Inventors: J.C. Herr and R.M. Wright
Title: "Primate Intra-Acrosomal Sperm Antigen for Use in a Contraceptive Vaccine"
UVAPF Docket no. 0215-05 (Herr-Sprmagp)
Filed Oct. 30, 1995
U.S. Patent 5,753,231 Issued May 19, 1998

7. Inventors: J. C. Herr and R. M. Wright
Title: "Human Sperm Diagnostic"
UVAPF Docket no. 215-13 (Herr-Sprmdia)
Filed April 25, 1994
U.S. Patent 5,605,803, Issued Feb. 25, 1997.

8. Inventors: J.C. Herr, A.B. Diekman, E. Norton, and A. Westbrook-Case
Title: "Purified Sperm Surface Antigen, Monoclonal Antibody Therefor and Applications Therefor"
UVAPF Docket No. 216-01 (Herr-Sprmdia)
Filed June 28, 1996
U.S. Patent 5,830,472 Issued Nov. 3, 1998.

9. Inventors: J.C. Herr, A.B. Diekman, V.A. Westbrook-Case, and E. Norton
Title:"Purified Sperm Surface Antigen, Monoclonal Antibody Therefor and Applications Therefor"
UVAPF Docket No. 216-03 (Herr-Sprmdia)
Filed Feb. 23, 1998
U.S. Patent 6,258,364 Issued July 10, 2001

10. Inventors: P.P. Reddi, CJ. Flickinger, J.C. Herr

Title: "Methods and Composition for Modulating Spermatogenesis"
UVAPF Docket no. 00271-04 (Herr-Mouse)
Filed June 25, 1999
U.S. Patent 6,355,480 Issued on March 12, 2002

11. Inventors: Z. Hao, J.C. Herr, F. Jayes, J. Shetty, and M. Wolkowicz
Title: "Sperm Specific Proteins"
UVAPF Docket no. 00497-08 (Herr-NIH)
PCT/US01/01717 filed Jan. 19, 2001
Pending U.S. Divisional Application no. 10/809,654 (off of 10/181,642 Filed March 25, 2004)
U.S. Patent 6,924,121 Issued on Aug 2, 2005

12. Inventors: J.C. Herr, P. Visconti, and Z. Hao
Title: "Human Testis Specific Serine/Threonine Kinase 1&2"
US Patent 6,946,275 B2
Issued Sept. 20, 2005
UVAPF Docket no. 00623-07 (Herr-Kinase)
PCT/US01/46803 Filed Nov. 9, 2001
U.S. Application no. Filed May 9, 2003

13. Inventors: J.C. Herr, S.C. Coonrod, and P. Wright
Title: "Egg-Specific Surface Proteins"
US Patent 6,962,988
Issued November 8, 2005
UVAPF Docket no. 00498-07 (Herr-MOP/CD9)
PCT/US01/01718 Filed Jan. 19, 2001
Pending U.S. Application no. 10/181,612 Filed July 18, 2002

B. Issued Foreign Patents

14. Inventors: J.C. Herr and R.M. Wright
Title: "Human Intra-Acrosomal Sperm Antigen for Use in a Contraceptive Vaccine"
UVAPF Docket nos. 0215-08-10, 12, 19-30 (Herr-Sprmagp)
16 Foreign Patents Issued in Denmark (461177), Norway (312594), Canada (2,0505,910), Australia (649609), Sweden (461,177), Spain (461177), Switzerland (461177), France (461177), Austria (461177), Germany (461177), United Kingdom (461177), Luxembourg (461177), Netherlands (461177), Italy (461177), Belgium (461177), and Liechtenstein (461177)

15. Inventors: J.C. Herr, A.B. Diekman, E. Norton, and V.A Westbrook-Case
Title: "Purified Sperm Surface Antigen, Monoclonal Antibody Therefor and Applications Therefor"
UVAPF Docket nos. 216-05-7, 10, 15, 16 (Herr-SprmAb1)
6 Foreign Patents Issued in Israel (127,764), Czech Republic (293040), Australia (722559), Eurasian Patent Office (199900067K), Poland (186809), Slovakia (281505)

16. Inventors: J.C. Herr, S. Naaby-Hansen, and C. Flickinger
Title: "Method for the Production of Vaccines Against Cell Surface Proteins"
UVAPF Docket no. 00217-04 (Herr-Vocprod)
1 Foreign Patent Issued in Australia (743703) Issued May 16,2002

C. Pending U.S. National Stage Patent Applications

15. Inventors: J.C. Herr, K. Klotz, and A. Diekman
Title: "Sperm Cell Selection Systems for Forensic DNA Analysis of the Male Component"
UVAPF Docket no. 00474-03 (Herr-Bead)
PCT/US00/31771 Filed Nov. 17, 2000
Pending U.S. Application no. 10/146,552 Filed May 15, 2002

16. Inventors: J.C. Herr, A. Mandal, F. Jayes, J. Shetty, and M. Wolkowicz
Title: "Sperm Specific Lysozyme Like Proteins"
UVAPF Docket no. 00489-08 (Herr-Lysozym)
PCT/US01/01716 Filed Jan. 19, 2001
Pending U.S. Application no. 10/181,611 Filed July 18, 2002

17. Inventors: J.C. Herr, S. Naaby-Hansen, M. Wolkowicz, A. Mandal, and Sen Beur
Title: "CBP86, a Sperm Specific Protein"
UVAPF Docket no. 00492-07 (Herr-CBP86)
PCT/USO1/01715 Filed Jan. 19, 2001
Pending U.S. Application no. 10/181,638 Filed July 19, 2002

18. Inventors: J.C. Herr. E. J. Norton, and A. B. Diekman
Title: "Recombinant Antibody Directed Against the Human Sperm Antigen"
UVAPF Docket no. 00415-03 (Herr-RASA)
PCT/US00/19843 Filed July 21, 2000
Pending U.S. Application Nationalized no. 10/031,783 Filed May 2, 2002

19. Inventors: J.C. Herr, J. Shetty, M. Wolkowicz, F. Jayes, and Z. Hao
Title: "Sperm Specific Proteins"
UVAPF Docket no. 00497-07 (Herr-NIH)
PCT/US01/01717 filed Jan. 19, 2001
Pending U.S. Application Nationalized no. 10/181,642 Filed March 25, 2004

20. Inventors: Z. Hao, J.C. Herr, F. Jayes, J. Shetty, and M. Wolkowicz
Title: "Sperm Specific Proteins"
UVAPF Docket no. 00497-08 (Herr-NIH)
PCT/US01/01717 filed Jan. 19, 2001
Pending U.S. Divisional Application no. 10/809,654 (off of 10/181,642 Filed March 25, 2004)

21. Inventors: Z. Hao, J.C. Herr, F. Jayes, J. Shetty, and M. Wolkowicz
Title: "Sperm Specific Proteins"
UVAPF Docket no. 00497-09 (Herr-NIH)
PCT/US01/01717 filed Jan. 19, 2001
Pending U.S. Divisional Application no. 10/809,655 (off of 10/181,642 Filed March 25, 2004)

22. Inventors: J.C. Herr, P. Visconti, and Z. Hao
Title: "Human Testis Specific Serine/Threonine Kinase 1&2"
UVAPF Docket no. 00623-07 (Herr-Kinase)
PCT/US01/46803 Filed Nov. 9, 2001
Pending U.S. Application no. 10/438,339 Filed May 9, 2003

23. Inventors: J.C. Herr, P. Visconti. Z. Hao, and G. Kopf
Title: "Human Testis Specific Serine/Threonine Kinase 3"
UVAPF Docket no. 00623-012 (Herr-Kinase3)
PCT/US01/46803 Filed Nov. 9, 2001
Pending U.S. Application no. 10/438,339 Filed May 9, 2003

24. Inventors: J.C. Herr, A. Mandal, M. Wolkowicz, and K. Klotz.
Title: "Methods and Compositions for Modulating Fertility"
UVAPF Docket no. 00121-03 (Herr-FSP95)
PCT/US00/02675 Filed Feb. 1, 2000
Pending U.S. Application no. US 09/890,709 Filed July 31, 2001

25. Inventors: J.C. Herr and P.P. Reddi
Title: "An Insulator Element Having Enhancer-Blocking Properties"
UVAPF Docket no. 00567-03 (Herr-Silence)
PCT/US01/17110 filed on May 25, 2001
Pending U.S. Application no. US 10/297,008 Filed Nov. 26, 2002

26. Inventors: J.C. Herr, P.E. Visconti, A. Wagenfield, M.A. Coppola, Z. Hao, and S. Vemuganti.
Title: "TSSK4, A Human Testes Specific Serine/Threonine Kinase"
UVAPF Docket no. 00927-02 (Herr-Kinase4)
Pending U.S. Application no. 10/754,829 Filed Jan. 8, 2004

D. Pending Foreign National Stage Patent Applications

27. Inventors: J.C. Herr and R.M. Wright
Title: "Human Intra-Acrosomal Sperm Antigen for Use in a Contraceptive Vaccine"
UVAPF Docket no. 0215-11 (Herr-Sprmagp)
PCT/US90/00978 Filed March 2, 1990
Pending Foreign Application Nationalized in Japan

28. Inventors: J.C. Herr, A.B. Diekman, and E. Norton.
Title: "Purified Sperm Surface Antigen, Monoclonal Antibody Therefor and Applications Therefor"
UVAPF Docket nos. 216-11-14,15,16 (Herr-SprmAb1)
PCT/US97/10813 Filed June 30, 1997
Pending Foreign Applications Nationalized in Japan, Ukraine, Brazil, Norway, Republic of Korea, Canada, Mexico, China, and Hungary

29. Inventors: J.C. Herr, S. Naaby-Hansen, and C. Flickinger
Title: "Method for the Production of Vaccines Against Cell Surface Proteins"
UVAPF Docket no. 00217-05-12 (Herr-Vocprod)
PCT/US98/02913 Filed Feb. 25, 1998
Pending Foreign Applications Nationalized in Brazil, Canada, Hungary, Israel, Japan, Mexico, Poland, and the Republic of Korea

30. Inventors: J.C. Herr, S.C. Coonrod, and P. Wright.
Title: "Egg-Specific Surface Proteins" filed Jan. 20, 2000.
UVAPF Docket no. 00498-03-6 (Herr-MOP/CD9)
PCT/USO1/01718 Filed Jan 19, 2001
Pending Foreign Applications Nationalized in Australia, Canada, European Patent Office, and Japan

31. Inventors: P.P. Reddi, CJ. Flickinger, and J.C. Herr
Title: "Methods and Composition for Modulating Spermatogenesis"
UVAPF Docket no. 00271-05-09 (Herr-Mouse)

PCT/US99/14275 Filed June 25, 1999
Pending Foreign Applications Nationalized in Israel, European Patent Office, Japan, Australia, and Canada

32. Inventors: J.C. Herr, A. Mandal, F. Jayes, J. Shetty, and M. Wolkowicz
Title: "Sperm Specific Lysozyme Like Proteins"
UVAPF Docket no. 00489-04-07 (Herr-Lysozym)
PCT/US01/01716 Filed Jan. 19, 2001
Pending Foreign Applications Nationalized in Australia, Canada, European Patent Office, and Japan

33. Inventors: J.C. Herr, S. Naaby-Hansen, A. Mandal, S. Beur, and M.J. Wolkowicz
Title: "CBP86, a Sperm Specific Protein"
UVAPF Docket no. 00492-03-06 (Herr-CBP86)
PCT/USO1/01715 Filed Jan. 19, 2001
Pending Foreign Applications Nationalized in Australia, Canada, European Patent Office, and Japan

34. Inventors: J.C. Herr, J. Shetty, M. Wolkowicz, F. Jayes, and Z. Hao
Title: "Sperm Specific Proteins"
UVAPF Docket no. 00497-03-06 (Herr-NIH)
PCT/US01/01717 Filed Jan. 19, 2001
Pending Foreign Applications Nationalized in Australia, Canada, European Patent Office, and Japan

35. Inventors: J.C. Herr, P. Visconti, and Z. Hao
Title: "Human Testis Specific Serine/Threonine Kinase 1&2"
UVAPF Docket no. 00623-04-06,-08 (Herr-Kinase)
PCT/US01/46803 Filed Nov. 9, 2001
Foreign Applications Nationalized in Australia, Israel, Japan, and the European Patent Office

36. Inventors: J.C. Herr, P. Visconti. Z. Hao, and G. Kopf.
Title: "Human Testis Specific Serine/Threonine Kinase 3"
UVAPF Docket no. 00623-09-11,-13 (Herr-Kinase3)
PCT/US01/46803 Filed Nov. 9, 2001
Foreign Applications Nationalized in Australia, Israel, Japan, and the European Patent Office

37. Inventors: J.C. Herr, A. Mandal, M. Wolkowicz, and K. Klotz.
Title: "Methods and Compositions for Modulating Fertility"
UVAPF Docket no. 00121-03 (Herr-FSP95)
PCT/US00/02675 Filed Feb. 1, 2000
Foreign Application Nationalized Australia (35868/00)

E. Pending PCT Applications

38. Inventors: J.C. Herr, M.B. Herrero, A. Mandal, and L.C. Digilio
Title: "Sperm Specific Lysozyme-Like Proteins"
UVAPF Docket no. 00857-02 (Herr-SLP.USE)
PCT/US04/01240 Filed Jan. 16, 2004

39. Inventors: C.D. Allis, J.C. Herr, S.A. Coonrod, and Y. Wang
Title: "ePAD, an Oocyte Specific Protein"
UVAPF docket no. 00856-02 (Herr-ePAD)
PCT/US04/00591 Filed Jan. 8, 2004

40. Inventors: J.C. Herr, P.E. Visconti, A. Wagenfield, M.A. Coppola, Z. Hao, and S. Vemuganti
Title: "TSSK4, A Human Testes Specific Serine/Threonine Kinase"
UVAPF Docket no. 00927-02 (Herr-Kinase4)
PCT/US04/02531 Filed Aug. 5, 2004

41. Inventors: J.C. Herr and A. Mandal
Title: "SpermCollect™, A Glans Compatible Single Unit Semen Collection and Storage Device, Kit, and Related Method Thereof"
UVAPF Docket no. 00942-02 (Herr-Collect)
PCT/US04/036916 Filed Nov. 5, 2004

42. Inventors: John C. Herr and Susan Sleight
Title: "Bands5: A Human Testis Specific Protein"
UVAPF Docket no. 00955-02 (Herr-Band5)
PCT/US04/041440 Filed Dec. 8, 2004

43. Inventors: L. Gilmer, A. Mandal, M.J. Wolkowicz, K.L. Klotz, and J.C. Herr
Title: "Compositions of Identifying Sperm for Forensic Applications"
UVAPF Docket no. 00952-03 (Herr-Paint)
PCT (no. to be assigned) Filed Feb. 7, 2005

F. Pending Provisional Patent Applications

44. Inventors: A. Mandal and J.C. Herr
Title: "Active Recombinant Human Lysozyme"
UVAPF Docket no. 00954-01 (Herr-ActiveLZ)
Provisional Filed Feb. 20, 2004

45. Inventors: Y.H. Kim and J.C. Herr
Title: "SFEC, A Sperm Flagellar Energy Carrier Protein"
UVAPF Docket no. 00973-01 (Herr-SFEC)
Provisional Filed March 17, 2004

46. Inventors: S.H. Lim, J.C. Herr, and A. Mandal
Title: "Use of SLLP Proteins for Identification, Diagnosis, and Treatment of Cancer"
UVAPF Docket no. 01000-01 (Herr-SLLPCAN)
Provisional Filed June 22, 2004

47. Inventors: S.B. Sleight, J.C. Herr, and B. Xu
Title: "Sperm Specific Raft Associated Proteins"
UVAPF Docket no. 01035-01 (Herr-Band10)
Provisional Filed Aug. 25, 2004

48. Inventors: J.C. Herr and Y.H. Kim
Title: "Sperm Flagellar Energy Carrier Protein as a Contraceptive Target"
UVAPF Docket no. 01045-01 (Herr-Polyol)
Provisional Filed Sept. 30, 2004

49. Inventors: J.C. Herr, B. Xu, and Z. Hao
Title: "Validation of TSSK Family Members and TSKS as Male Contraceptive Targets"
UVAPF Docket no. 01048-01 (Herr-TSSK)
Provisional Filed Sept. 30, 2004

50. Inventors: Y.H. Kim and J.C. Herr
Title: "SFEC, A Sperm Flagellar Energy Carrier Protein"
UVAPF Docket no. 00973-01 (Herr-SFEC)
Provisional Filed March 17, 2004

51. Inventors: J.C. Herr, V.A. Westbrook, P.D. Schopeee, and K.L. Klotz
Title: "Use of SPAN-X for Identification, Diagnosis and Treatment of Fertility and Cancer Related Diseases and Disorders"
UVAPF Docket no. 01059-01 (Herr-XAssay)
Provisional Filed Nov. 5, 2004

52. Inventors: W. He and J.C. Herr
Title: "Compositions and Methods for Novel ePAD Genes"
UVAPF Docket no. 01063-01 (Herr-cdePAD)
Provisional Filed Nov. 23, 2004

53. Inventors: W. He and J.C. Herr
Title: "Contraceptive Vaccines for Dogs and Cats Based on Egg Membrane Antigens"
UVAPF Docket no. 01065-01 (Herr-VetVaccine)
Provisional Filed Nov. 23, 2004

54. Inventors: O. Chertihin and J.C. Herr
Title: "Contraceptive Vaccines for Dogs and Cats Based on Egg Membrane Antigens"
UVAPF Docket no. 01065-02 (Herr-Vet2)
Provisional Filed Dec. 14, 2004

Other Patents

55. Inventors: D. Gerdt and J.C. Herr. "Fiber Optic Evanescent Wave Sensor for Immunoassay." US Patent #5,494,798, Granted Feb. 27, 1996.

Abstracts

1. Azzam, N. A., and J. C. Herr 1976 Surface anatomy of the rat median eminence. A scanning and transmission electron microscopic study. Second Annual College of Medicine Research Day, University of Iowa.

2. Azzam, N. A., and J. C. Herr 1976 Correlative SEM-TEM study of the rat median eminence and hypothalamic wall of the third ventricle. Soc. Neuroscience, Sixth Annual Meeting, Toronto, p. 644.

3. Herr, J. C. 1977 Decidual cells in the human ovary at term: incidence and ultrastructure. American Association of Anatomists, 90th Annual Session, Detroit, Michigan. Anat. Rec. 187: p. 603.

4. Herr, John C. 1978 Decidual cells in the human ovary at term: freeze-fracture features. American Association of Anatomists, 91st Annual Session, Vancouver, B. C. Anat. Rec., 190: p. 421.

5. Herr, J. C., A. C. Hahnel, and E. M. Eddy 1979 Analysis of sperm surface components using specific antisera and gel electrophoresis. American Association of Anatomists, 92nd Annual Session, Miami. Anat. Rec., 193: p. 565.

6. Herr, J. C., A. C. Hahnel, and E. M. Eddy 1979 Mouse sperm surface antigens identified with heterologous antiserum and immunoabsorption. Am. Soc. Cell Biol., p. 228.

7. Herr, J. C., and E. M. Eddy 1980 Evidence for a sperm component acquired in the vas deferens. American Association of Anatomists, 93rd Annual Session, Omaha. Anat. Rec., 196: p. 77A.

8. Herr, J. C., C. H. Muller, B. F. Fendersen, and E. M. Eddy 1981 Topographically restricted isoantigens of mouse sperm present on spermatogenic cells. American Association of Anatomists 94th Annual Session, New Orleans. Anat. Rec., 199: 113A.

9. Herr, J. C. 1982 Calmodulin in bovine epididymal fluid. American Society for Cell Biology. J. Cell Biol., 95: 173a.

10. Kisalus, L., W. Nunley, and J. C. Herr 1983 Co-distribution of SP1 and HPRL by immunocytochemical localization in human syntrophoblast. Society for Study of Reproduction 1983, Biol. Reprod. p. 119A.

11. Brown, B. L., B. A. Smith, F. S. Baechtel, and J. C. Herr 1984 Identification of human spermatozoa by indirect immunofluorescence using polyclonal antiserum. American Academy of Forensic Sciences, Feb. 1984, Anaheim, Calif.

12. Herr, John C., Jackson E. Fowler, William M. Sutherland, Deborah J. Koons, and Mark Sigman 1984 Anti-sperm monoclonal antibodies from human X human hybridomas constructed postvasectomy. Anat. Rec. 208, 3: 73A.

13. Bruns, M. E., E. Kleeman, S. E. Mills, J. Herr, and D. E. Bruns 1984 Cellular localization of vitamin D-dependent intestinal calcium binding protein in small intestine, yolk sac, placenta and uterus of pregnant mice. Calcified Tissue International 36, 511.

14. Sigman, M., W. M. Sutherland, B. L. Brown, and J. C. Herr 1984 Anti-human sperm monoclonal antibodies from mouse X mouse hybridomas. ASBC/AAI, St. Louis, June 3-7, 1984. Federation Proceedings, 43, 6: 1707.

15. Sigman, M., R. S. McGee, W. M. Sutherland, and J. C. Herr, 1984 Electrophoretic analysis of human sperm antigens recognized by mouse monoclonal antibodies. International Symposium on Forensic Applications of Electrophoresis, June 1984, FBI Academy, Quantico, Virginia

16. McGee, R. S., T. L. Rankin, M. Sigman, B. Brown, J. C. Herr, and B. Baer 1984 Analysis of human antigenic determinants by immunofluorescence and western blot with mouse polyclonal antisera. International Symposium on Forensic Applications of Electrophoresis, June 1984, FBI Academy, Quantico, Virginia.

17. Rankin, T. L., B. Brown, R. S. McGee, S. Baechtel, and J. C. Herr 1984 Evidence for multiple antigenic determinants using anti-prostatic-antigen specific on Forensic Applications of Electrophoresis, June 1984, FBI Academy, Quantico, Virginia.

18. Herr, John C., Teresa A. Summer, Tracy L. Rankin, William M. Sutherland and Robert S. McGee 1985 Monoclonal antibody MHS-5 recognizes low MW human seminal fluid antigens - a marker for primate semen. J. Androl. Vol. 6 March/April p. 54.

19. Bruns, M. E., E. Kleman, S. E. Mills, D. E. Bruns and J. Herr 1985 Immunochemical localization of vitamin D-dependent CABP in mouse placenta and yolk sac. Federation Proceedings 44, #3, p. 1153.

20. Herr, John C., Lydia L. Kisalus and Donald R. Spell 1985 Protein synthesis and ultrastructural analysis of human decidual cells in vitro. Anat. Rec. 211, 82A.

21. Herr, J. C., R. S. McGee, and R. J. Evans 1985 A novel sperm-coating antigen originating in the human seminal vesicles: evidence for binding to the human sperm surface using immunoblots, Elisa, and immunofluorescence using monoclonal antibody MHS-5. Proceedings XII Int. Congress of Anatomy, London, p. 283.

22. Flickinger, C. J., J. C. Herr and K. E. Ertl 1986 Epididymal luminal proteins of the mouse. Anat. Rec. 214, #3: 39A.

23. Riad, N. H., J. C. Herr, M. E. Bruns and N. H. Fares 1986 Ultrastructural localization of vitamin D-dependent calcium binding protein in the murine intraplacental yolk sac. Anat. Rec. 214, #3: 108.

24. Fares, Nagui Hansan, Mahmoud Ahmed Banhawy and John C. Herr 1986 Decidual cells in the human ovary at term: II Morphometric analysis of cytoplasmic process and organelles. Anat. Rec. 214, #3: 37A.

25. Kisalus, Lydia L., Charles D. Little and John C. Herr 1986 Identification of collagen types I, III, IV, V, fibronectin, laminin and intermediate filaments in human decidua. Anat. Rec. 214, #3: 66A.

26. McGee, Robert S. and John C. Herr 1986 Molecular weight transformation of a human seminal vesicle specific protein during semen liquefaction: Detection with monoclonal antibody MHS-5. Ant. Rec. 214, #3: 79A.

27. Herr, J. C., C. J. Flickinger and D. R. Spell Temporal appearance of antisperm autoantibodies in Lewis rats following vasectomy. J. Reprod. Immunol. 9, (Suppl 1), 83.

28. Herr, J. C., R. S. McGee and D. J. Conklin 1986 Purification of seminal vesicle specific antigen by immunoaffinity chromatography on bound monoclonal antibody MHS-5. International Symposium on Forensic Immunology. June 25, 1986.

29. Kisalus, L. L. and J. C. Herr 1986 Protein synthesis in human decidua of early pregnancy. J. Cell Biol., 103, p. 486a (1816).

30. Flickinger, C. J., J. C. Herr, K. L. Klotz and K. E. Ertl 1986 Major luminal proteins of the mouse epididymis: isolation and production of antiserum. J. Cell Biology, 103, p. 482a (1801).

31. Herr, J. C. and M. W. Woodward 1986 An Elisa assay for human semen identification using a monoclonal antibody to a seminal vesicle specific antigen. International Symposium on Forensic Immunology. FBI Academy June 25.

32. Flickinger, C. J., J.C. Herr, S. S. Howards, D. Spell and D. Caloras 1987 Testicular alterations and antisperm antibodies in Lewis rats after vasectomy and vasovasostomy. Anat. Rec., 218: 46A.

33. Herr, J. C. and D. J. Conklin 1987 Purification of seminal vesicle specific antigen by cation exchange and immunoaffinity chromatography on bound monoclonal antibody MHS-5. Anat. Rec., 218: 56A.

34. Handley, H. H., J. C. Herr and Charles J. Flickinger 1987 Sperm cell autoimmunogens following vasectomy in the Lewis rat. Anat. Rec. 218, #1: 56A.

35. Scott, J. A., D. C. Benjamin, C. A. Scott, J. C. Herr, V. H. Engelhard 1987 Effects of neonatal transplantation of MHC-compatible thymuses into diabetes - prone BB rats. Diabetes, 36, Suppl 1, 65A.

36. Scott, J. A., V. H. Engelhard, C. A. Scott, J. C. Herr and D. C. Benjamin 1987 Neonatal transplantation of MHC-compatible thymuses into diabetes-prone BB rats. Proceedings of European Association for Study of Diabetes.

37. Herr, J. C., C. J. Flickinger, K.L. Klotz and J. Foster 1988 A polymorphic acrosome specific antigen recognized within human sperm by monoclonal antibody MHS-10. J. Androl. 9, #1, p. 43.

38. Herr, J.C., C. J. Flickinger, D. R. Spell, D. J. Conklin 1988 Immunoelectron microscopic localization of seminal vesicle specific antigen in human seminal vesicle. Anat. Rec., 220:43A.

39. Christie, C.M., V.H. Engelhard, D.C. Benjamin, J.C. Herr and J. Scott 1988 Effects of thymus grafts on granulocytes in the diabetes-prone BB rat. Diabetes 37 (Suppl 1), 210.

40. J. Scott, C.M. Christie, V.H. Engelhard, J.C. Herr, S.E. Adams, D.C. Benjamin 1988 Effects of neonatal thymectomy and thymus grafting on polymorphonuclear neutrophils and eosinophils in diabetes-prone BB rats. Internal Diabetes Federation, Sydney, Australia (Nov. 88).

41. Handley, H.H, J.C. Herr, C.J. Flickinger 1988 Temporal fluctuations in autoantibody to defined rat sperm antigens following vasectomy Anat. Rec., 220:42A.

42. Handley, H.H., J.C. Herr, C.J. Flickinger 1988 Specificity of autoantiserum eluted from PAGE transblot separated rat sperm autoantigens. J. Reprod. Immunol. 16, #3, p. 109.

43. M. Homyk, D.J. Anderson, H. Wolf, J.C. Herr 1989 A novel immunohistochemical probe for differential diagnosis of immature germ cells during semen analysis. Am. J. Reprod. Immunol. 19, 75A.

44. H.H. Handley, C.J. Flickinger, and J.C. Herr 1989 Immunohistochemical localization of Lewis rat post-vasectomy autoimmunogens in spermatozoa and testis. J. Reprod. Immunol., 15 (Suppl 10), 104.

45. R.M. Wright, J.E. John, C.J. Flickinger, and J.C. Herr 1989 Cloning and characterization of cDNAs coding for the human sperm acrosomal protein, SP-10. J. Cell Biol. 109: #4, 66a.

46. J.C. Herr, R.M. Wright, J. Foster, and C.J. Flickinger 1989 Species cross-reactivity of monoclonal antibody and cDNA probes for human acrosomal antigen SP-10: Identification of primate and pig models. J. Cell Biol. 109: #4. 65a.

47. D.L. Fulgham, D. Johnson, C.C. Coddington, J.C. Herr, N.J. Alexander, G.D. Hodgen 1989 Human sperm acrosome reaction rate on the zona pellucida: A time course study. Am. Fertility Soc. Abstract prog, suppl, p. S45.

48. J.A. Foster and J.C. Herr 1990 Studies of the human sperm intra-acrosomal protein SP-10 following treatments with Triton X-114 and sodium thiocyanate. Anat. Rec. 226, #4: 33A.

49. B. Kurth, K. Klotz, J.C. Herr, C. Flickinger 1990 Localization of sperm antigen SP-10 during the seminiferous cycle in man. Anat. Rec. 226, #4: 54A.

50. J.C.Herr, R.M. Wright, R.L. Eddy, T.B. Shows 1990. The gene for the intra-acrosomal human sperm antigen SP-10 maps to chromosome 11, specifically to the p12 q13 region. Am. J. Reprod Immunol. 22: 91a.

51. M. Homyk and J.C.Herr 1991 Immunolocalization of human sperm antigens by light and electron microscopy. J. Androl., Jan/Feb Suppl p. 37.

52. J.A. Foster and J.C. Herr 1991 Partial Characterization of SP-10 interactions with human acrosomal membranes. J. Andrology, Jan/Feb Suppl, p. 44.

53. H.R. Brashear, M.P. Vigilone, Y.I. Kim, L. Li, J.C. Herr 1991. Occurrence of antineuronal antibody without encephalomyeloneuritis in a patient with paraneoplastic Lambert-Eaton Syndrome. Am. Neurology 30, #2, p. 310.

54. J.A. Foster, J.C.Herr 1991 Triton X-114 Studies of human sperm protein SP-10 and its association with the acrosomal membranes. Am. J. Reprod. Immunol. 25, #2, p. 56a.

55. K. L. Beecher, M. J. Homyk, C. Y. G. Lee, J.C. Herr 1991. Characterization of a sperm protein recognized by monoclonal antibody HSA-1. Am J. Reprod. Immunol. 25, #2, p. 56a.

56. J.C. Herr, R.M. Wright, C.J. Flickinger, R.L. Eddy, T.B. Shows 1991 Assignment of the gene for human intra-acrosomal protein SP-10 {ACRV1} to the p12->q13 region of chromosome 11. Cytogenetics and Cell Genetics 58: #3-4, p. 1963.

57. A. J. Freemerman, J.C. Herr and Richard M. Wright 1991 Cloning and sequencing of baboon SP-10: A comparison of sequence and amino acid homology between human and baboon SP-10. Am. J. Reprod. Immunol. 25, #2, p. 63a.

58. B. Kurth, R. Wright, J. Herr and C. Flickinger 1992 Detection of sperm protein SP-10 mRNA during the seminiferous cycle in man by in situ hybridization. Anat. Rec. 232, #4: 53A.

59. A.J. Freemerman, R.M. Wright and J.C. Herr 1992 Cloning and sequencing of primate SP-10: A comparison of sequence and amino acid homology between human and primate SP-10. J. Androl. 13 [suppl]: p.40A.

60. J.A. Foster, K.L. Klotz, R.M. Wright, J.R. Castillo, J.C. Herr 1993 Electron microscopic immunolocalization and western blot analysis of SP-10 in human sperm from the male reproductive tract and following ejaculation, J. Androl. Supplement January/February 1993, p. 28.

61. J.C. Herr, R.M. Wright, K. Klotz, M. Homyk, B. Kornreich, C.J. Flickinger, J. Powell, V. Stevens 1993 Immunogenicity of SP-10 fusion proteins in female baboons, J. Immunol. 150, #8, p. 16a.

62. J.C. Herr and Dale L. Laux 1994 Characterization of a monoclonal antibody cocktail to the human sperm head antigen SP-10: a novel immunostaining procedure for sperm identification. Proceedings Am. Acad. Forensic Sci, p. 93.

63. M. Wolkowicz and J.C. Herr 1994 Testis-specific gene expression during acrosomal biogenesis. Am. J. Reprod. Immunol. 31: p. 225-226.

64. P.P. Reddi, J.Y. Tsai, R. Bollag, L. Silver, J.C. Herr 1994 Molecular cloning and genomic localization of mSP-10, a murine homologue for human acrosomal protein, SP-10. Am. J. Reprod. Immunol. 31: p. 225.

65. S. Segal, D. Koul, K. Vashist, J.C. Herr 1994 Serum antibodies to recombinant human intra-acrosomal antigen SP-10 in infertile couples. Am. J. Reprod. Immunol. 31: p. 239.

66. L.A. Baker, T.T. Turner, J.C. Herr 1994 Absence of endocrinological and immunological support for a contralateral effect after experimental unilateral testicular torsion. Abstracts for 1994 Annual Meeting of the Society for Basic Urological Research.

67. S. Naaby-Hansen, J.C. Herr 1995 A 2-D protein database for the human spermatozoa. J. Androl., Jan/Feb Supplement, p. 27.

68. S.A. Coonrod, J.C. Herr 1995 SP-10 is present in bovine sperm and antibodies to SP-10 inhibit bovine fertilization in vitro. Am. J. Reprod. Immunol. 33 (6): p. 442.

69. S. Naaby-Hansen, J. Shannon, and J.C. Herr 1995 Identification of calreticulin in human spermatozoa by microsequencing from 2D gels. Mol. Biol. Cell, Vol. 6, p. 429a.

70. M.J. Wolkowicz and J.C. Herr 1995 Analysis of the GC-2spd cell line for expression of cell type-specific markers of differentiation. Mol. Biol. Cell, Vol. 6, p. 315a.

71. V.A. Westbrook-Case, A.B. Diekman, V.V. Khole, and J.C. Herr, 1995. Characterization of the acrosomal matrix protein P71/72 in human spermatozoa. Mol. Biol. Cell, Vol. 6, p. 428a.

72. C.M Vidaeus, C. Kap-Herr, W. Golden, R.L. Reddy, T.B. Shows, and J.C. Herr, 1996. Cloning and characterization of a candidate contraceptive vaccinogen, human fertilin. Am. J. Rep. Immunol., Vol. 35, p. 466a.

73. B.E. Kurth, C.D. Weston, R. Bhattacyarya, C.J. Flickinger, J.C. Herr 1996 Specific antibodies in oviductal fluid after intramuscular immunization of *Macaca fascicularis* with a recombinant sperm antigen, Am. J. Rep. Immunol, Vol. 35., p. 467a.

74. M.S. Dubova-Mihailova, K.L. Klotz, M.L. Baran, C.J. Flickinger, J.C. Herr 1996 Identification, molecular cloning and characterization of sperm tail auto-antigen SA-ODF75. 36th American Society for Cell Biology Annual Meeting, December 7-11, 1996, San Francisco, CA.

75. Reddi, P.P., C.J. Flickinger, P. Sassone-Corsi, J.C. Herr 1997 Promoter analysis of a testis specific gene which encodes the acrosomal protein, SP-10. Testis Workshop, 1997.

76. Herr, J.C., S. Naaby-Hansen, M.J. Wolkowicz, H. Shibahara, D. Koul, A. Mandel, J. Shetty, C.J. Flickinger 1997 Strategies for the development of a sperm antigen based contraceptive vaccine. ESHRE 13th Annual Meeting, June 22-25, 1997, Edinburg, Scotland. Human Reproduction 12 (Abstracts), p. 63.

77. Naaby-Hansen, S., M. Wolkowicz, J. Shannon, M. Kinter, N.E. Sherman, P.P. Reddi, H. Shibahara, L.A. Bush, J. Fox, J.C. Herr 1997 Human sperm calreticulin: from two-dimensional gel spot to isolation of testicular cDNA. Human Reproduction 12 (abstract book 1), p. 117.

78. Shibahara, H., S. Naaby-Hansen, S.A. Coonrod, J. Shetty, C.J. Flickinger, J.C. Herr 1997 Identification of glycosylphosphatidylinositol (GPI)-anchored human sperm membrane proteins. Society for the Study of Reproduction 30th Annual Meeting.

79. Naaby-Hansen, S., M.J. Wolkowicz, H. Shibahara, J. Shetty, C.J. Flickinger, J.C. Herr 1997 Towards a comprehensive 2-D gel protein database for the human spermatozoon. Society for the Study of Reproduction 30th Annual Meeting, Portland, Oregon. Biology of Reproduction, 56:172, Supplement 1 (Abs. 358) (1997).

80. Kurth, B., J. Srinivasan, R. Bahttacharya, C. Flickinger, J. Herr, R. Curtiss 1997 Recombinant avirulent *Salmonella* does not induce antigen-specific immune responses in cynomolgus monkeys. Immunol. Cell Biol. 75 (suppl 1):A56.

81. Herr, J.C., S. Naaby-Hansen, A.B. Diekman, M.J. Wolkowicz, A. Westbrook-Case, E. Norton, K. Klotz, H. Shibahara, D. Koul, A. Mandal, J. Shetty, C.J. Flickinger 1997 Discovery and characterization of sperm contraceptive vaccinogens. "Vaccines Beyond 2000", CSIRO, Australia.

82. Norton, E.J., A.B. Diekman, V. A. Westbrook, K.L. Klotz, and J.C. Herr 1998 Immunohistochemical localization of a carbohydrate epitope in the human reproductive tract: Implications for spermicidal development. The FASEB Journal 12:#5, A1104.

83. Wolkowicz, MJ, S. Naaby-Hansen, and J.C. Herr 1997 Cloning, sequencing, expression and analysis of a human testicular tektin B1-like protein. Biology of Reproduction.58 [Supp 1]:175.

84. Coonrod, S.A., S. Naaby-Hansen, J. Shetty, H. Shibahara, and J.C. Herr. 1998 Treatment of zona-free hamster occytes with PI-PLC blocks human sperm-hamster egg interaction and releases a protein cluster (33-43 kDa, PI 4.3-5.5) from the hamster oolemma. Bio. Reprod. 58 [Supp 1]: 157.

85. Shetty, J., S. Naaby-Hansen, H. Shibahara, R. Bronson, C.J. Flickinger and J.C. Herr 1998 Immunodominant sperm antigens recognized by sera from infertile men and women containing antisperm antibodies. Biol. Reprod.58 [Supp 1]: 131.

86. Reddi PP, Flickinger CJ, and Herr J.C. 1998 Minimal promoter essential for round spermatid-specific gene expression resides within the -420 to +28 BP region of the SP-10 gene. Biol. Reprod. 58 (Supp 1):163.

87. V.A. Westbrook, A.B. Diekman, K.L. Klotz, S. Naaby-Hansen, W. Lai and J.C. Herr 1998 Characterization and localization of the human sperm protein nap-x. Mol. Biol. Cell 9: 66a [suppl]

88. A. Mandal, S. Naaby-Hansen, K. Klotz, M.J. Wolkowicz, J. Shetty, F. Cesar, C.J. Flickinger and J.C. Herr 1998 Two novel human sperm fibrous sheath proteinswhich undergo phosphorylation during capacitation. Mol. Biol. Cell (suppl. 9:178a)

89. S.A. Coonrod, S. Naaby-Hansen, J. Shetty, H. Shibahara, M. Chen, D. Bigler, J.M. White and J.C. Herr 1998 PI-PLC releases protein clusters (70 kDa, pI 5;35-45 kDa,pI 5.5) from the mouse oolemma and blocks sperm-egg binding and fusion. Mol. Biol. Cell 9: 311a

90. M.S. Chen, S.A. Coonrod, Y. Takahashi, Y. Yamashita, P.W. Kincade, K.S. Tung, J.C. Herr, and J.M. White 1998 Role of the integrin-associated protein CD9 in sperm-egg binding and fusion. Mol. Biol. Cell 9: 295a

91. Hiroaki Shibahara, Eiko Wakimotol, Soren Naaby-Hansen, Jagathpala Shetty, John C. Herr, and Koji Koyama 1999 Identification of major immunodominant human sperm antigens recognized by sera from infertile women with sperm-immobilizing antibodies (SI-Abs) using two-dimensional (2-D) gel electro- phoresis. Biology of Reproduction 60: [Supplement 1]: 257.

92. K. Kshitish Acharya, P. Prabhakara Reddi and John C. Herr 1999 Dissection of the SP-10 promoter and identification of testis-specific DNA-protein interactions. Biology of Reproduction 60 [Supplement]: 212.

93. J.C. Herr, Kenneth Klotz, Peggy Anderson, Edward Adams, Norman Moore and Stuart S. Howards Immunochromatographic card for detecting human sperm. Gynecological Urological Surgeons Meeting. AAGUS, April 15, 1999.

94. John C. Herr, Kenneth Klotz, Peggy Anderson, Edward Adams, Norman Moore and Stuart Howards 1999 SpermCheck immunochromatographic card. American Urological Association, Inc. May 5, 1999.

95. Scott Coonrod, Michael Kinter, Nicholas Sherman, Soren Naaby-Hansen, Jagathpala Shetty, John Herr, 1999 Identification of mouse egg surface proteins by tanden mass spectrometry. Biology of Reproduction 60: [Supplement 1]: 208.

96. Elizabeth J. Norton, Alan B. Diekman, V. Anne Westbrook, Kenneth L. Klotz, John C. Herr 1999 RASA: A recombinant anti-sperm antibody directed against the sperm glycoform of CD52. Mol. Biol. Cell [Supplement] 359a.

97. R.M. Turner, M. Musse, J.C. Herr, G. L. Gerton, S.B. Moss, and H.E. Chemes 2000 Molecular genetic analysis of two fibrous sheath proteins, AKAP4 and AKAP3 in men with dysplasia of the fibrous sheath. J. Androl. Suppl. P46:48

98. Prabhakara P. Reddi and John C. Herr 2000. A 436 -basepair proximal promoter of the mouse SP-10 gene, which activates spermatid-specific transcription in vivo, functions as a repressor in somatic cell-lines. Biol. Reprod.62 (Suppl 1): 290.

99. Jagathpala Shetty, Alan Diekman, Friederike Jayes, Nicholas Sherman, Soren Naaby-Hansen and John C. Herr 2000. Differential extraction and enrichment of human sperm surface proteins in a 2-D proteome: Identification of immunocontraceptive candidates. Biol. Reprod).62 (Suppl 1): 152.

100. Coonrod SA, Chen MS, Reddi PP, Diekman AB, Kasper EN, Thompkins TR, White JM and Herr JC 2000. Plasma membrane localization of mouse ZP3. Biol. Reprod. 62 (Suppl 1): 167.

101. Tod C. McCauley, Alan B. Diekman, Elizabeth J. Norton, Barbara E. Kurth, V. Anne Westbrook and John C. Herr. 2000 Analysis of sperm agglutination antigen-1 (SAGA-1), A glycoprotein implicated in human infertility, in non-human primates. Biol.Reprod.62 (Suppl 1): 154.

102. Zhonglin Hao, Michael J. Wolkowicz, Jagathpala Shetty, Charles J. Flickinger and John C. Herr. 2000 Cloning and characterization of putative human sperm membrane antigen related to circumsporozoite (SMARC32). Biol. Reprod. 62 (Suppl 1):154.

103. Kshitish K. Acharya, John C. Herr and Prabhakara P. Reddi. 2000 Testis-specific 47kDA nuclear protein, a putative transcription factor, binds to single and double stranded sp-10 promoter elements. Biol. Reprod. 62 (Suppl 1): 229.

104. Anne Westbrook, Alan B. Diekman, Scott A. Coonrod, Ken L. Klotz, Sen Buer and John C. Herr. 2000 Characterization of span-x in the mammalian testis and transfected cells. Biol. Reprod. 62 (Suppl 1): 334.

105. Prabhakara P. Reddi, Joshua A. Shapiro, Amy N. Shore, Kshitish K. Acharya and John C. Herr. 2000 Dual role for the proximal promoter of the SP-10 gene: enchancer in spermatids and insulator in all other cell-types. Mol. Biol. Cell (Suppl)11: 310a.

106. Zhonglin Hao, Scott Coonrod, Amy Shore, Charles J. Flickinger and John C. Herr. Cloning of TACC3 from the mouse egg proteome. Frontiers in Reproduction Boston, MA June 2000.

107. A.B. Diekman, R.J. Norton, T.C. McCauley, K.L. Klotz, V.A. Westbrook, B.E. Kurth, P. Anderson and John C. Herr. The use of carbohydrate epitopes in immunocontraceptive development. 6th European Training Course on Carbohydrates, 2000.

108. Shetty, J., Wolkowicz, M.J., Diekman, A., Jayes, F.L., Flickinger, C.J. and Herr, J.C. 2000 Cloning and characterization of C58, a novel hydrophobic, putative human sperm membrane antigen related to the Ly-6 family of proteins. Second International Conference on Experimental and Clinical Reproductive Immunobiology 2000: 90.

109. A.B. Diekman, E.J. Norton, T.C. McCauley, V.A. Westbrook, P. Anderson, B.E. Kurth, K.L. Klotz, R. Easton, A. Dell, H. Morris and J.C. Herr. 2000 The use of carbohydrate epitopes in immunocontraceptive development. Second International Conference on Experimental and Clinical Reproductive Immunobiology 2000: 93.

110. Arabinda Mandal, Ken Klotz, Michael J. Wolkowicz, Jagathpala Shetty, Friederike L. Jayes, Charles J. Flickinger and John C. Herr. 2000 APL17 and APLX: Two novel human acrosomal pseudo-lysozymes. Mol. Biol. Cell (Suppl)11: 520a.

111. Wolkowicz, M.J., Shetty, J., Jayes, F., Flickinger, C.J. and Herr, J.C. An autoantigenic osteoglycin-like sperm protein discovered by 2D proteomics. Second International Conference on Experimental and Clinical Reproductive Immunobiology 2000: 88.

112. Soren Naaby-Hansen, Arabinda Mandal, Michael J. Wolkowicz, Buer Sen, Jacque Retief, V. Anne Westbrook, Jagathpala Shetty, Michael Cox, Friederika Jayes, Scott A. Coonrod, Kenneth L. Klotz, Leigh Ann Bush, Charles J. Flickinger and John C. Herr Alternative splicing of CBP86, a novel testis-specific CA^{2t} -binding protein localized in the tail of human sperm. Second International Conference on Experimental and Clinical Reproductive Immunobiology 2000: 89.

113. K.K. Acharya, J.C.Herr, and P.P. Reddi Molecular cloning of 3 putative testicular transcription factors that bind to a 38 mer region in the promoter of the spermatid-specific gene SP-10. International Congress on Fertilization,Embryo Development and Implantation 2000: 41.

114. Prabhakara P. Reddi, Amy N.Shore, Joshua A. Shapiro, Kshitish K. Acharya and John C. Herr Regulation of germ cell-specific gene expression: Insights from the study of acrosomal protein SP-10. International Congress on Fertilization, Embryo Development and Implantation 2000: 12.

115. John C. Herr, Soren Naaby-Hansen, Arabinda Mandal, Michael J. Wolkowicz, Buer Sen, Jacque Retief, V. Anne Westbrook, Jagathpala Shetty, Michael Cox, Friederika Jayes, Scott A. Coonrod, Kenneth L. Klotz, Leigh Ann Bush, and Charles J. Flickinger. Proteomic approaches to identify genes uniquely expressed in the testis: CBP86, a novel testis-specific calcium binding protein localized in the tail of human sperm undergoes phosphorylation and oligomerization during capacitation. International Congress on Fertilization, Embryo Development and Implantation 2000: 10.

116. Arabinda Mandal, Soren Naaby-Hansen, Michael J. Wolkowicz, Sen Buer, Jacque Retief, V. Anne Westbrook, Jagathpala Shetty, Michael Cox, Friederika Jayes, Scott A. Coonrod, Kenneth L. Klotz, Leigh Ann Bush, Charles J. Flickinger, and John C. Herr. 2000 CBP86, A novel testis-specific Ca^{2+}-binding protein localized in the human sperm tail is regulated by phosphorylation during *in vitro* capacitation. Mol. Biol. Cell 11:52a.

117. Pablo E. Visconti, Zhonglin Hao, Marie Purdon, Paula Stein, Binaifer R. Balsara, Joseph R. Testa, John C. Herr, Stuart B. Moss and Greg Kopf. 2000 Cloning and chromosomal localization of a novel serine/threonine kinase belonging to the subfamily of testis-specific serine/threonine kinases tssk. Mol. Biol. Cell 11: 521a.

118. Shetty, J., Wolkowicz, M.J., Diekman, A., Jayes, F.L., Thompkins, T.R., Flickinger, C.J. and Herr, J.C. Cloning and characterization of C58, a novel, hydrophobic, putative human sperm membrane antigen related to the Ly-6 family of proteins. National Merit Fellowship, Bristol-Myers Squibb Pharmaceutical Research Institute, Hopewell, NJ. March 8, 2001

119. Scott Ficarro, Olga Chertihin, Forest M. White, Jeffrey Shabanowitz, John C. Herr, Pablo Visconti and Donald F. Hunt. Phosphoproteome analysis of capacitated human sperm cells.

120. Michael A. Coppola, V. Anne Westbrook, Pamela D. Schoppee and John C. Herr. Use of SPAN-X, a New Cancer-Testis Antigen, as a Tumor Marker._21st Annual Seminar of Cancer Research in Virginia, Richmond, VA. April 7, 2001.

121. J.C. Herr, J. Shetty, M.J. Wolkowicz, Z. Hao, S. Naaby-Hansen, A. Mandal, F. F. Jayes and C.J. Flickinger. 2001 Sperm-specific auto- and iso-antigens identified by 2-D gel base proteomics. Am. J. Rep. Immunol, Vol. 46., p. 71.

122. Prabhakara, P. Reddi, Amy N. Shore, Kshitish Acharya, and John C. Herr. 2001 Evidence that a transcriptional insulator ensures programmed gene expression of SP-10 during spermatogenesis. Biol. of Reprod. Vol. 64., p.194.

123. Zhonglin Hao, Michael J. Wolkowicz, Jagathpala Shetty, Ken Klotz, Scott A. Coonrod, Laura Bolling, Sen Buer, V. Anne Westbrook, Charles J. Flickinger and John C. Herr. 2001 SAMP 32, A novel testis-specific, membrane-associated protein, is localized to the acrosome of human spermatozoa. Biol.of Reprod. Vol. 64., p.194.

124. Clara I. Marin-Briggiler, Olga Chertihin, Kenneth L. Klotz, John C. Herr, Monica Vazquez-Levin and Pablo Visconti. Calcium/calmodulin-dependent protein kinase IV is present in human sperm and is involved in the regulation of sperm motility. The Gordon Conference, Fertilization and Activation of Development, New Hampshire, July 29-August 3, 2001.

125. Elizabeth V. Berkeley, Margaret C. Shea, Paul W. Wright, Laura C. Bolling, John C. Herr, and Scott Coonrod. Preliminary Investigations into Identification of Cat Oocyte-Specific Proteins. Alliance for Contraception in Cats and Dogs, Callaway Gardens, Georgia. April 19, 2002.

126. Visconti, PE, Ficarro S, Chertihin, O, White, F, Westbrook, A, Kalab P., Shababowitz, J, Herr, J.C. and Hunt, D. 2002 Phosphoproteome analysis of Capacitated Human Sperm Cells. J. of Androl. 23 (2) Supplement p. 51.

127. J. Herr, J. Shetty, S. Naaby-Hansen, A. Mandal, M.J. Wolkowicz, Z. Hao, S. B. Sen, J. Retief, V. Westbrook, F. Jayes, S. Coonrod, K. Klotz, L. Bush, M. Coppola and C. Flickinger. 2002 Sperm Proteomics and the discovery of Targets for Contraception and Cancer. Am J. Reprod. Immunology. 47 (6): p. 340.

128. Arabinda Mandal, Kenneth L. Klotz, Jagathpala Shetty, Friederike L. Jayes, Michael J. Wolkowicz, Laura C. Bolling, Scott A. Coonrod, Michael B. Black, Alan B. Diekman, Timothy AJ Haystead, Charles J. Flickinger and John C. Herr. 2002 SLLP1, A novel non-bacteriolytic c lysozyme-like protein in the acrosome of Human Spermatozoa. Biol. of Reprod. 60: (7) Supplement p. 113.

129. Olga Sarmento, Laura Bolling, Paul Wright, Yanming Wang, David Allis, John Herr and Scott Coonrod. 2002 Resetting the "Histone Code" in the Mammalian Ovum: A "Proof-of-Principle" Study.

130. Kshitish K. Acharya, Amy N. Shore, Chhabi Govind, Craig Urekar, John C. Herr, and Prabhakara P. Reddi. 2002 Spermatid-Specific Promoter Functions As Insulator in Somatic Cells. October 2002. "Germ Cells" at Cold Spring Harbor Laboratories.

131. Maria B. Herrero, Laura C. Digilio, Arabinda Mandal, Scott Coonrod, and John C.Herr. 2002 An intra-acrosomal c lysozyme-like protein 1, mSLLP1, mediates sperm-oolemma binding in the mouse. J. of Andrology, March/April 2003 supp. P55

132. Wolkowicz, M.J., Shetty, J., Westbrook, A., Klotz, K., and Herr, J.C. 2002 Equatorial Segment Protein (ESP): An alloantigenic glycoprotein provides evidence for an equatorial segment subcompartment in acrosomal vesicle stage spermatids. Experimental Biology Meeting, San Diego, CA. Abs. #215.7/3819.FASEB Journal 17:A356 (April 2003).

133. J.C. Herr, C.J. Flickinger, J. Shetty, A. Westbrook, L. Bolling, K. Klotz, S. Coonrod, F. Jayes, A. Mandal and M. Wolkowicz. 2003 Definition of the equatorial segment as a discrete sub-compartment in the nascent acrosomal vesicle that persists throughout human acrosomal biogenesis. Testis Workshop, Phoenix, Arizona p. 35

134. J.C. Herr, M.J. Wolkowicz, J. Shetty, A. Mandal, Z. Hao, L. Digilio, and B.E. Kurth. Immunogenicity of a pentavalent recombinant subunit anti-acrosomal vaccine in monkeys. Hippokration Congress on reproductive Immunology. Rhodes, Greece. (June, 2003).

135. Kula N. Jha, Olga Chertihin, Sangeeta Kumari, Criag Foster, John C. Herr, and Pablo E. Visconti. 2003 Screening of protein kinases present in mammalian sperm: evidence for a proline directed phosphorylation cascade involved in capacitation. Society for the Study of Reproduction 36th Annual Meeting, Cincinnati, Ohio. Biol. Reprod. 68:p118, #17.

136. Arabinda Mandal, Laura C. Digilio, Maria B. Herrero, Theodore S. Thomas, Charles J. Flickinger, and John C. Herr. 2003 Human SLLP1 binds to the perivitelline matrix and plasma membrane of mouse, hamster, and human eggs. Society for the Study of Reproduction 36th Annual Meeting, Cincinnati, Ohio. Biol. Reprod. 68:p120, #22.

137. Prabhakara Reddi, Amy N. Shore, Chhabi K. Govind, Melissa C. Stokes, Mark H. Stoler, John C. Herr, and Kshitish K. Acharya. 2003 Two acacac motifs in the proximal promoter are critical for the spatiotemporal expression of the SP-10 gene during spermatogenesis. Identification of cognate transcription factors. 2003 Society for the Study of Reproduction 36th Annual Meeting, Cincinnati, Ohio. Biol. Reprod. 68:p219, # 261.

138. Jayasimha Rao, Charles J. Flickinger, Prabakara P. Reddi, Mark H. Stoler, Amy Shore, Dirk R. Yonkee, Leigh Ann Bush, and John C. Herr. 2003 Region-specific expression of sperm associated defensin-and lectin-like iso-antigen (E-3) in the rat epididymis. 2003 Society for the Study of Reproduction 36th Annual Meeting, Cincinnati, Ohio. Biol. Reprod. 68:p300, #458.

139. Young-Hwan Kim, Arabinda Mandal, Erin Farris, Charles J. Flickinger, and John C. Herr. 2003 CABYR splice variants utilizing coding region A (CR-A) and coding region B (CR-B) are expressed in sperm and calcium binding occurs to isoforms containing only CR-A. 2003 Society for the Study of Reproduction 36th Annual Meeting, Cincinnati, Ohio. Biol. Reprod. 68:p 350, # 577.

140. Geeta Vanage, Ken Klotz, Young-Hwan Kim, Kula N. Jha, Olga Chertihin, John Herr 2003

Immunocytochemical Localization of HumanGlutathione S-transferase m3 and Mouse Acrosomal Protein12 in Spermatozoa. Fogarty: International Research Training Program in Population and Health & International Maternal and Child Health Research Training Program Joint Network, December 4-5, 2003 Washington, DC

141. Young-Hwan Kim, Kula Nand Jha, Arabinda Mandal, Erin Farris, Michael Wolkowicz, Charles J. Flickinger, and John C. Herr. 2003 CABYR splice variants utilizing coding region A (CR-A) and coding region B (CR-B) are expressed in sperm and calcium binding occurs to isoforms containing only cR-A" Fogarty: International Research Training Program in Population and Health & International Maternal and Child Health Research Training Program Joint Network, December 4-5, 2003 Washington, DC

142. Vrinda Khole, Arabinda Mandal, Leigh Ann Bush, Anne Westbrook, Pablo Visconti, John C. Herr 2003 Localization of Testicular sAC in Human Sperm and Evidence of Multiple Splicing in the Gene and Potential Protein Heterogeneity" Fogarty: International Research Training Program in Population and Health & International Maternal and Child Health Research Training Program Joint Network, December 4-5 2003 Washington, DC

143. Kula Nand Jha, Olga Chertihin, Sangeeta Kumari, Craig Foster, John C. Herr and Pablo E. Visconti 2003 Screening of Protein Kinases Present in Mammalian Sperm; Evidence for a Proline-directed Phosphorylation Cascade Involved in Capacitation" Fogarty: International Research Training Program in Population and Health & International Maternal and Child Health Research Training Program Joint Network, December 4-5, 2003 Washington, DC

144. Fralix, Kimberly D., Anderson, P.C., Pulido, S., Diekman, A.B., Dell, A., and Herr,J.C. 2003 Molecular Approaches to Vaccine Design: Characterization of the Carbohydrate Moieties of a Unique CD52 Glycoform Found on Human Spermatozoa: A Candidate Contraceptive. 2003 Cold Spring Harbor Conference, Dec. 2003, New York.

145. B. Xu, M.A. Coppola, M.P. Timko, J.C. Herr. Expression of A Recombinant Anti-sperm Mini-plantibody in Tobacco. 2003 The American Society for Cell Biology 43rd Annual Meeting, San Francisco, California. Mol. Biol. Cell. 14:525a.

146. P.D. Schoppee, J. Rolle, J.C. Herr. Localization of SPAN-X Protein During the Cell Cycle in Cancer Cells Lines. 2003 The American Society for Cell Biology 43rd Annual Meeting, San Francisco, California. Mol. Biol. Cell. 14:245a.

147. Maria Belen Herrero, Arabinda Mandal, Laura Digilio, John Christian Herr. 2004 A unique c-lysozyme-like protein has a role in fertilization. FASEB, April 2004

148. Geeta R. Vanage, Jagathpala Shetty, Kenneth L. Klotz, Vivian A. Westbrook, Charles J. Flickinger, and John C. Herr. 2004 Localization of SAMP14, A Novel Acrosomal Membrane Associated Protein, During Acrosome Biogenesis in the Human Testis. Society for the Study of Reproduction 37th Annual Meeting, Vancouver, Canada. Biol. Reprod. 39:p162, #304.

149. Arabinda Mandal, Kenneth L. Klotz, Jagatphala Shetty, Soumya A. Vemuganti, Michael A. Coppola, Timothy A.J. Haystead, Charles J. Flickinger, and John C. Herr. 2004 SLLP2, A

Novel Non-Bacteriolitic Lysozome-Like protein of Human Spermatozoa Linked to the X Chromosome. Society for the Study of Reproduction 37th Annual Meeting, Vancouver, Canada. Biol. Reprod. 39:p125, #143.

150. Young-Hwan Kim, Buer Sen, Geeta Vanage, Arabinda Mandal, Michael J. Wolkowicz, Silvia Pulido, Kenneth Klotz, Zhonglin Hao, Charles J. Flickinger, and John C. Herr. 2004 Polymorphic CABYR Coding Region A (CR-A) abd Coding Region B (CR-B) are Expressed in Mouse Sperm and Localize to the Fibrous Sheath. Society for the Study of Reproduction 37th Annual Meeting, Vancouver, Canada. Biol. Reprod. 39:p252, #695

151. Zhonglin Hao, Bingfang Xu, Young-Hwan Kim, Laura Digilio, Silvia Pulido, Anne Westbrook, Mark Stoler, Pablo Visconti, Charles Flickinger, and John C. Herr. 2004 Gene Expression Studies of Two Members of the Mammalian Testis Specific Serine/Threonine Kinase Subfamily (TSSK2, TSSK4) and a Substrate (TSKS). Society for the Study of Reproduction 37th Annual Meeting, Vancouver, Canada. Biol. Reprod. 39:p186, #409.

152. Maria Herrero, Laura Digilio, Jennifer Monroe, John Herr, and Prabhakara Reddi. Functional Assays Demonstrate that the Mouse Acrosomal Protein SP-10 Plays a Role In Sperm-Zona Binding. 2004 Society for the Study of Reproduction 37th Annual Meeting, Vancouver, Canada. Biol. Reprod. 39:p 23, #52.

153. John C. Herr and Young-Hwan Kim 2004 Proteomics of Isolated Human Sperm Fibrous Sheath: Machinery of Glycolysis and a Novel Sperm Flagellar Energy Carrier Protein. The Future of Male Contraception Meeting, Seattle, WA. Sept. 29-Oct. 2, 2004.

154. John C. Herr, Kenneth L. Klotz, Stuart S. Howards, Christina Wang, Edward Leary, Kyung-ah Kim and Jemo Kang 2004 SpermCheck® Contraception: An Immunochromatographic Cassette for Monitoring Male Contraceptive Efficacy. The Future of Male Contraception Meeting, Seattle, WA. Sept. 29-Oct. 2, 2004.

155. Bingfang Xu, Zhonglin Hao, Craig Urekar, Kula N. Jha, Young-Hwan Kim, Laura Digilio, Geeta R. Vanage, Kenneth Klotz, Soumya Vemuganti, Anne Westbrook, Olga Chertihin, Jagathpala Shetty, Michael Coppola, Pablo Visconti, Charles J. Flickinger, John C. Herr 2004. Validation of the TSSK Family of Male Contraceptive Targets. The Future of Male Contraception Meeting, Seattle, WA. Sept. 29-Oct. 2, 2004.

156. Bingfang Xu, Zhonglin Hao, Craig Urekar, Kula N. Jha, Young-Hwan Kim, Laura Digilio, Geeta R. Vanage, Kenneth Klotz, Soumya Vemuganti, Anne Westbrook, Olga Chertihin, Jagathpala Shetty, Michael Coppola, Pablo Visconti, Charles J. Flickinger, John C. Herr 2004. Validation of TSKS as a Male Contraceptive Target. The Future of Male Contraception Meeting, Seattle, WA. Sept. 29-Oct. 2, 2004.

157. B. Xu, S.B. Sleight, M.J. Wolkowicz, S.M. Pulido, P. Anderson, P.D. Shoppee Bortz, L. Digilio, C.J. Flickinger, J.C. Herr. A Novel Flagella Putative Protease, SPRV1-2, Similar To Polycythemia Rubra Vera. XXVII North American Testis Workshop, Seattle , WA March 30-April 2, 2005.

158. YH Kim and JC Herr The Fibrous Sheath is a Compartment for Glycolysis. 25th Annual Meeting American Society Reproducitve Immunology June 18, 2005 Am. J. Rreprod. Immunol. 53, 6, 306 [ASRI05-78].

159. W He and JC Herr Identification and Cloning of the Dog Pad VI Gene Homologue. 25th Annual Meeting American Society Reproducitve Immunology June 18, 2005 Am. J. Reproductive Immunology 53, 6, 303 [ASRI05-68].

160. J.C. Herr, K.L. Klotz, S.S. Howards, K. Kim, J. Kang. 2005 Sensitivity of an Immunochromatographic Cassette for Monitoring the Presence of Human Sperm. Fertility and Sterility 84: S170.

161. J. C. Herr, L. C. Digilio, K. N. Jha, O. I. Chertihin. 2005 MOEP19: A Novel Mouse Oocyte and Early Embyo Protein Associated with Cortex and Perivitelline Matrix is Phosphorylated in vitro by Casein Kinase II and Protein Kinase A. Fertility and Sterility 84: S380.

Invited Presentations at Universities, Federal Agencies, Corporations, and National Meetings

"Isolation of Sperm Surface Antigens," Developmental Biology Retreat, University of Washington, February 10, 1979, Lake Wilderness.

"Sperm Surface Antigens," E. M. Eddy and J. C. Herr, Gordon Research Conference on the Mammalian Genital Tract. July 7-11, 1980, Colby-Sawyer College, New London, NH.

"Dissection of the Mouse Sperm Surface Using Heterologous and Monoclonal Anti-sperm Antibodies," University of Minnesota, June 9, 1980.

"Molecular Dissection of the Mouse Sperm Surface with Monoclonal Antibodies," Bowman-Gray Medical School, February 1981.

"Human Hybridomas Constructed with Peripheral Blood Lymphocytes from Vasectomized Men" Joint Immunobiology Meeting. Harpers Ferry, W.Va., May 13, 1984.

"Monoclonal Antibodies - New Dimensions in Immunological Analysis" May 18, 1984, Eli Lilly Sponsored, St. Cloud, Minnesota Hospital Forum.

"Monoclonal Antibodies in Forensic Stain Characterization" FBI Academy, June 11, 1984.

"Monoclonal Antibodies to Seminal Fluid Specific Marker Protein MHS-5 and Serum Albumins" FBI Academy, Nov. 13-14, 1984.

"MHS-5 - a Monoclonal Antibody for Forensic Science" Endotronics, Minneapolis, Minnesota, May 17, 1985.

"Monoclonal Antibodies and Forensic Serology" Scotland Yard, London, England, August 19, 1985.

"ELISA Assay for Human Semen in Forensic Samples Employing Monoclonal Antibody MHS-5" FBI Headquarters, Washington, D.C. September 12, 1985.

"Monoclonal Antibody Based Assays for Identification of Human Blood and Semen" Workshop on Allotype Genetic Markers, American Academy of Forensic Sciences, New Orleans, February 14, 1986.

"A Monoclonal Antibody Probe for Rape Detection" Charlottesville, Rape Crisis Center, February 19, 1986.

"Monoclonal Antibody Probes for Detection of Semen and Identification of Blood" October 17, 1986 Food and Drug Administration, Silver Spring, Maryland, Office of Medical Devices.

"Monoclonal Antibody, MHS-5 - A New Probe for Sexual Assault Evidence", Department of Biology, Virginia Commonwealth University, Richmond, VA. November 6, 1986.

"Monoclonal Antibody for Detection of Semen and Identification of Blood". Crime Lab Directors, FBI Academy, Quantico, VA, November 13, 1986.

"Application of Anatomy and Immunology in Criminology" Forum in Anatomy, May 11, 1987. American Association of Anatomy, Washington, D.C. Annual Meeting.

"Seminal Vesicle Specific Antigen" October 8, 1987 University of New Mexico, Albuquerque, NM.

"Monoclonal Antibodies to Cervical Mucus, Sperm Surface and Seminal Fluid". FBI Academy, Quantico, VA. February 11, 1988.

"Update on Human Decidual Secretion and Differentiation Antigens of Human Spermatogenesis". Genentech, Inc., South San Francisco, CA. February 19,1988.

"Novel Antigens Involved in Human Semen Liquefaction and Spermatogenesis". University of Iowa, Iowa City, IA. February 26, 1988.

"Monoclonal Antibody Probe for Novel Seminal Vesicle Specific Marker - New Diagnostic for Rape Detection". Igen, Inc., Rockville, MD. March 7, 1988.

"Overview of FBI Contract Research at the University of Virginia". Invited Speaker for FSOPC Committee, May 3, 1988, FBI Academy.

"Human Sperm Surface and Acrosomal Antigens Probed with Murine Monoclonal Antibodies". Invited Symposium Speaker, Symposium on Sperm Antigens in Reproduction, Am. Assoc. for Immunol. of Reproduction, Portland, ME. June 17, 1988.

"Biochemical, Morphological and Genetic Characterization of SP-10 an Intra-Acrosomal Human Sperm Protein" The Population Council, The Rockefeller Univ., New York, N.Y. March 16, 1989.

"Molecular Biology of Human Spermatogenesis - Characterization of the intra-acrosomal antigen SP-10." Dept. Anatomy, University of Iowa, May 26, 1989.

"Molecular Biology of Human Spermatogenesis - Characterization of the intra-acrosomal antigen SP-10 and its encoding gene." Dept. Anatomy, Univ. of Wisconsin-Madison, May 30, 1989.

"The Intra-acrosomal antigen SP-10" 4th International Congress of Reproductive Immunology, Kiel, Germany, July, 1989.

"Contraceptive Vaccine Candidate SP-10" Ortho Pharmaceuticals, Raritan, NJ, August 11, 1989.

"A Marker for Sperm Heads in Sexual Assault Evidence". Lifecodes Corp, Valhalla, NY, August 22, 1989.

"Monoclonal Antibody MHS-10 and its Cognate Antigen SP-10" CONRAD Symposium of Immunocontraception, Bariloche, Argentina, November, 1989.

"Biochemical and Morphological Characterization of SP-10" National Institute of Immunology, New Delhi, India, March 14, 1990

"Biochemical and Morphological Characterization of the Intra-acrosomal antigen SP-10" May 11. 1990 National Institute of Environmental Health Sciences, Research Triangle, N.C.

"A Differentiation Antigen of Human Spermatogenesis" Gordon Research Conference, Mammalian Genital Tract, Wolfboro, NH July 11, 1990.

"Development of A Sperm Based Contraceptive Vaccine" Food and Drug Administration, Rockville, MD, Jan 29, 1991.

"Contraceptive Vaccine Candidate SP-10 Associated with Human Sperm Acrosomal Membranes" American Association for the Advancement of Science, Washington, D.C., Feb 15, 1991.

"Biochemical, Morphological and Immunogenicity Studies of a Sperm Based Contraceptive Vaccine for Women" Louisiana State University, New Orleans, LA. March 18, 1991.

"Organization and Direction of the Center for Recombinant Gamete Contraceptive Vaccinogens" National Institutes of Health, Lister Hall, Washington, D.C., Feb. 27, 1992.

"Intra-Acrosomal Contraceptive Vaccine Immunogen SP-10 in Man, Macaque and Baboon" Serono Symposium, Beaverton, OR. May 31, 1992.

"Update on Sperm Antigen SP-10" 20th Anniversary Symposium of World Health Organization Special Program of Research, Development and Research Training in Human Reproduction. Moscow, Russia, June 17, 1992.

"Cell and Molecular Biology of the Intra-acrosomal Antigen SP-10" Gordon Conference on Mammalian Genital Tract, Plymouth State College, Plymouth, NH, July 9, 1992.

"Antibodies to Sperm Protein SP-10 in Infertile Couples; The Expression of Pure Recombinant SP-10 to Serve as Assay Target" U.S.A.I.D. Contraceptive Development and Research Initiative Working Group. Washington, DC, September 17, 1992.

"Updates in Immunocontraception" Armed Forces District American College of Obstetrics and Gynecology, November 2, 1992, Norfolk, VA.

"Molecular Approaches to Gamete Surface Antigens" December 2, 1992, Calcutta, India.

"Biochemical, Morphological and Genetic Characterization of the Intra-Acrosomal Antigen SP-10" December 4, 1992, Institute of Chemical Biology, Calcutta, India.

"Immunogenicity of Recombinant SP-10 in Female Baboons" December 9, 1992, Symposium on Recombinant and Synthetic Vaccines, Delhi, India.

"Biochemical, Morphological, and Genetic Characterization of the Intra-Acrosomal Sperm Protein SP-10: A Human Contraceptive Vaccine Candidate Currently Undergoing Testing in Baboons" March 4, 1993, Oregon Regional Primate Research Center, Beaverton, OR.

"Sperm Surface Antigens as Targets for Immunocontraception" April 17, 1993, 18th Annual Meeting American Society of Andrology, Tampa, FL.

"Biochemical, Morphological and Genetic Characterization of Intra-Acrosomal Protein SP-10" May 11, 1993, Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC.

"Prospects for Contraceptive Vaccines Based on Sperm-Surface Immunogens: May 18, 1993, Grand Rounds Department Obstetrics and Gynecology, Oregon Health Sciences University, Portland, OR.

"Intra-Acrosomal Protein SP-10: A Contraceptive Vaccine Candidate" May 24, 1993. Reproductive Immunology Mini-Symposium, American Association for Immunology, Denver, CO.

"Immunologic Aspects and Potentials in Contraception" June 5, 1993, Thornton Conference, Omni Hotel, Charlottesville, VA.

"Immunogenicity of SP-10 Fusion Proteins in Female Baboons" June 18, 1993, Sixth Annual Mid-Atlantic Reproductive Biology Meeting, Johns Hopkins University, Baltimore, MD.

"The Immunobiological Effects of Vasectomy and Vasovasostomy in the Rat Model" August 29, 1993, Serona Symposium on Immunobiology of Reproduction, Boston, MA.

"Results of Baboon Immunogenicity Trials Comparing GT-SP-10 Fusion Protein from pGEX with SP-10 Produced in pET" October 29, 1993, NIH Contraceptive Development Centers Annual Meeting, Wintergreen, VA.

"Update on Contraceptive Vaccine Immunogen - SP-10" December 11, 1993, Japanese Society for Reproductive Immunology, Nara, Japan.

"Microheterogenicity of the Intra-acrosomal Protein SP-10 is due to Alternative Splicing and Proteolytic Processing" February 9, 1994, Boden Conference, Thredbo, Australia.

"Sperm Specific Intra-Acrosomal Antigen SP-10" May 12, 1994, Endocrinology and Reproductive Biology Seminar Series, The Population Council, The Rockefeller University, New York, NY.

"cDNA Cloning in Progress: Sperm Antigens Recognized by Auto and Iso Anti-Sperm Antibodies" June 3, 1994, Seventh Mid-Atlantic Reproductive Biology Meeting, Georgetown University, Washington, DC.

"Candidate Vaccinogens for a Sperm Based Immunocontraceptive" September 30, 1994, Zonagen, Inc., Woodlands, TX

"Overview of Contraceptive Vaccine Development in the Center for Recombinant Gamete Contraceptive Vaccinogens" December 8, 1994, Institute of Medicine, National Academy of Science, Washington, DC

"Human Sperm Surface Proteins as Contraceptive Vaccinogens" May 2, 1995, Department of Anatomy and Cell Biology, University of Iowa

"Biochemical, Morphological and Genetic Characterization of the Intra-Acrosomal Protein SP-10, a Contraceptive Vaccine Candidate" May 4, 1995, Seminar Series in Cell Biology, Developmental Biology and Neuroscience, University of Kansas, Kansas City

"A Tale of Sperm, Sperm Antigens and Infertility" May 5, 1995, Medical Histology and Cell Biology, University of Kansas Medical School

"Advances in Immunocontraception," June 5, 1995, National Advisory Committee on Child Health and Human Development, National Academy of Sciences, Washington, D.C.

"Immunizing with Sperm Antigens to Prevent Fertilization," July 8, 1995, SSR Workshop on the Immunology of Implantation, Davis, California.

"The Use of Acrosomal Antigens as Contraceptive Immunogens: Studies on the Intra-acrosomal Antigen SP-10," International Symposium on the Human Sperm Acrosome Reaction, September 9, 1995, Collioure, France

"Recent Advances on the Path to a Contraceptive Vaccine," 15th World Congress on Fertility and Sterility, September 20, 1995, Montpelier, France

"Morphological, Biochemical, and Genetic Characterization of the Intra-acrosomal Protein SP-10," December 5, 1995, Department Zoology, Ain Shaims University, Cairo, Egypt.

"The Biotechnology of Contraceptive Vaccine Development," December 9, 1995, Ain Shams University, Cairo, Egypt.

"The Path to Contraceptive Vaccine Development," December 11, 1995, Al-Azhar University, Cairo, Egypt.

"Development of a Home-Sperm-Test," February 2, 1996, Chefaro, Oss, The Netherlands.

"Strategies in the Development of a Sperm Based Contraceptive Vaccine," February 3, 1996, Organon, N.V., Oss, The Netherlands.

"Steps on the Pathway to a Contraceptive Vaccine," March 22, 1996, Finnish Andrology Society Plenary Lecture, Turku, Finland.

"The Development of Sperm Antigen Based Contraceptive Vaccines," March 25, 1996, Leiras, N.V. Turku, Finland.

"Sperm Antigens in Infertility and Contraception," June 1, 1996, Clinical Immunology Society, New Orleans, LA.

"Contraceptive Vaccine Development," October 11, 1996, Food and Drug Administration, Rockville, MD.

"The Two Cultures: The Language of Science and the Language of Venture Capital, Refining Scientific Ideas for the Venture Community," Conference on Opportunities for Industrial Collaboration in Contraceptive Research, Family Health International, November 7, 1996, Durham, NC.

"Multidisciplinary Training in Contraceptive Vaccine Development for Indian Postdocs," May 20, 1997, Advisory Board Fogarty International Center, National Institutes of Health, Washington, DC.

"Strategies for the Development of a Sperm Antigen Based Contraceptive Vaccine," June 24, 1997, European Society of Human Reproduction and Embryology, Edinburgh, Scotland.

"Discovery and Characterization of Sperm Contraceptive Vaccinogens," July 8, 1997, Vaccines Beyond 2000, Gold Coast, Queensland, Australia.

"Strategies for Identification of Cell Surface Vaccinogens," September 30, 1997, First International Conference on Experimental and Clinical Reproductive Immunobiology, Charlottesville, VA.

"A Strategy for Identification of Sperm Surface Vaccinogens," November 16, 1997, NIH Joint Centers' Meeting, Davis, CA.

"Contraceptive Products Under Development in the Center for Recombinant Gamete Contraceptive Vaccinogens," January 8, 1998, presented to NIH Advisory Council on Contraceptive Clinical Network.

"Home Sperm Test" Virginia Biotechnology Association, Richmond, VA. Biotechnology Park, March 23, 1998.

"A Strategy for Identification and Cloning of Novel Sperm Surface Immunogens" Guest Symposium of Amer. Assoc. Immunologists, San Francisco, Calif. April 19, 1998.

"A Strategy for Identification of Sperm Immunogenes Relevant to Infertility". Symposium on Clinical Implications in Reproductive Biology, Woods Hole, Mass, June 10, 1998.

"Identification of Sperm Specific-Marker Proteins for use in Sperm Detection and Quantification." Virginia Tech Biotechnology Center, Blacksburg, VA. April 23, 1999.

"Preparations for a Human Trial on LDHC-4: TT Chimeric Peptide Vaccine: Rationale for Trial and Response of FDA to IND" Schering AG, Berlin, FRG. May 27, 1999

"Oolemmal Vaccine Development: Oolemmal Proteomics" Schering AG, Berlin FRG May 28, 1999.

"An Immunochromotographic Card for Detecting and Quantificating Sperm", Frontiers in Reproduction, Marine Biological Laboratories, Woods Hole, MASS. June 10, 1999

"2-D Sperm Proteomics" Frontiers in Reproduction, Marine Biological Laboratories, Woods Hole, MA. June 11, 1999

"Progress in Developing an Immunochromatographic Device for Sperm Detection" Association of Medical Laboratory Immunologists, Bethesda, MD. July 10, 1999

"Cooperative International Research and Capacity Building in Reproduction and Contraception" Workshop on Global Research on Women and Children's Health, NICHD, Bethesda, MD. September 14, 1999

"Design of the Home Sperm Test" Workshop on Improving Acceptability Research, NICHD, Bethesda, MD. October 25, 1999.

"Assorted Arcana of Sperm Cytoarchitecture" University of Pennsylvania, Center for Research on Reproduction and Women's Health. October 27, 1999.

"Sperm Binding Magnetic Bead Employing Sperm Agglutination Antigen 1 [SAGA-1]" Future Directions Session, Fifth Annual CODIS User's Group Meeting, US Department of Justice, Federal Bureau of Investigation, Arlington, VA. November 19, 1999.

"Sperm Binding Magnetic Bead Employing Sperm Agglutination Antigen 1 [SAGA-1]" Cellmark, a division of Astra Zeneca, Oxford, England. November 22, 1999.

"RASA: A Recombinant Anti-Sperm Antibody Directed Against the Sperm Glycoform of CD52". "Pre-Clinical Topical Microbiocides Workshop" NIH, Bethesda, MD. January 13, 2000.

"Overview of the Program: The Contraceptive Vaccine Search for Testis Specific Genes and Proteins Opens Other Contraceptive Leads" Schering AG, Berlin, FRG. January 26-28, 2000.

"Calcium binding to CBP86, a novel testis-specific calcium-binding protein localized in the principal piece of human sperm, is regulated by phosphorylation during capacitation" Schering AG, Berlin, FRG. January 26-28, 2000.

"Update on the LDH-C4 Contraceptive Vaccine IND" Schering AG, Berlin, FRG. January 26-28, 2000.

"A Sperm Binding Bead for Forensic-DNA Analysis" Schering AG, Berlin, FRG, January 26-28, 2000.

"New Approaches to Contraception" Executive Council Planned Parenthood of the Blue Ridge, October 2, 2000.

"CBP86, a novel testis-specific calcium binding protein localized in the tail of human sperm undergoes phosphorylation and oligomerization during capacitation" International Congress on Fertilization, Embryo Development and Implantation. New Delhi, India, November 6, 2000.

"Discovery Driven Translational Research in Sperm Proteomics and Genomics" Department of Molecular Reproduction, Development and Genetics, and the Department of Biochemistry, Indian Institute of Science, Bangalore, India, November 10, 2000.

"Sperm Proteomics and the Discovery of Contraceptive Targets" Schering AG, Berlin, Germany, November 14, 2000

"Span-X as a Cancer-Testis Marker" Schering AG, Berlin, Germany, November 14, 2000

"Proteomic Approaches to Identifying Contraceptive Targets" Second International Conference on Experimental and Clinical Reproductive Immunobiology, Amsterdam, The Netherlands, November 16, 2000.

"From Basic Sperm Biology to Commercialization of a Diagnostic Device: Biotechnology Underlying Sperm Check I, an Immunochromatographic Card for Detecting Low Numbers of Sperm" The Burroughs Welcome Lecture, University of Guelph, Ontario, January 30, 2001.

"Commercializing Academic Research Through Startup Companies" University of Guelph's Business Development Office, January 30, 2001.

"The Development of Recombinant Miniantibodies as Targeting Vectors: Sperm Aggulutination Antigen 1: Looking Toward a New Generation of Spermicidal Agents" Department Biomedical Sciences, University of Guelph, Ontario, January 31, 2001.

"Proteomics: Discovery of Contraceptive Vaccines in the Post-Genomic Era." Department of Biomedical Sciences, University of Guelph, Ontario, February 1, 2001.

"Discovery of a Unique Sperm Surface Molecule - SAGA-1, and its use in Forensic Science" Randolph-Macon College, April 7, 2001.

"A Multi-Determinant Contraceptive Vaccine Based Upon Testis-Specific Human Sperm Head Antigens: Immunogenicity and Efficacy Trials in Monkeys" Schering, AG, May 8, 2001.

"Sperm Cell Selection System for Forensic DNA Analysis of Male Component" National Institute of Justice, Washington, D.C., June 7, 2001.

"Sperm Proteomics and the Discovery of Testis-Specific Contraceptive Targets" American Society for Immunology of Reproduction 21st Annual Meeting, Chicago, IL, June 10, 2001.

"Sperm Proteomics and the Discovery of Testis-Specific Contraceptive Targets" Frontiers in Reproduction Symposium, Cambridge, MA, June 30, 2001.

"Sperm Proteomics and the Discovery of Testis-Specific Contraceptive Targets" VIII Congress of the International Society of Reproductive Immunology, Opatia, Croatia, July 6, 2001.

"Sperm Proteomics and the Discovery of Testis-Specific Contraceptive Targets" The Ludwig Institute, New York, N.Y. December 4, 2001.

"Sperm Proteomics and the Discovery of Contraceptive Targets" Dept. Anatomy and Cell Biology, Univ. of Iowa, May 30, 2002.

"Sperm Proteomics and the Discovery of Targets for Contraception and Cancer" Plenary Session, XX11nd Annual Meeting American Society of Reproductive Immunology. Chicago, Ill. June 6, 2002.

"The Biotechnology Underlying Sperm Immunodiagnostics" National Institute of Health John F. Fogarty International Center, Washington, D.C., June 11, 2002.

"Sperm Cell Selection System for Forensic DNA analysis of the Male Component" Third Annual DNA Grantees Conference, National Institute of Justice, Washington, D.C., June 24, 2002.

"Defining the Sperm Proteome" Gordon Research Conference on Gametogenesis and Embryogenesis. Connecticut College, July 4, 2002.

"Contraceptive Vaccines and Spermistatic Mini-antibodies Targeting Sperm Surface Sugars" 2nd Annual Advances in Contraceptive Health Symposium" Boston, MA., July 20, 2002.

"The Sperm Check Immunodiagnostic" Princeton Bio Medi Tech, Princeton, N.J., September 17, 2002.

"A Case Study in the Transfer of University Intellectual Property to a Biotechnology Start-up" General Assembly Building, Joint Commission on Technology and Science, Richmond, VA, September 25, 2002.

"Specification of an Equitorial Segment Domain During Early Acrosomal Biogensis—A Susceptible Biological Process For Targeting A Male Contraceptive?" Schering AG, Berlin, Germany, December 6, 2002.

"Equitorial Segment Protein and Acrosomal Biogenesis", Dept. of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY., May 6, 2003.

"Immunogenicity of a pentavalent recombinant subunit anti-acrosomal vaccine in monkeys." Hippokration Congress on Reproductive Immunology, June 4, 2003, Rhodes, Greece.

"Novel Acrosomal Matrix Proteins ESP and SAMP 14: Roles in fertilization and Acrosome Biogenesis." Georgetown University, Dept. of Cell Biology, June 12, 2003.

"Sperm Cell Selection System for Forensic DNA Analysis of the Male Component" Fourth Annual DNA Grantees Workshop, Natl. Institute of Justice, Washington, DC. June 25, 2003.

"The Biotechnology Underlying Sperm Immunodiagnosis" Syntron Bio Research Inc., Carlsbad, CA, July 2, 2003

"Proteomics and the Discovery of Contraceptive Drug and Vaccine Targets" Institute of Medicine, National Academy of Sciences, New Frontiers in Contraceptive Research International Symposium, Washington, DC. July 15, 2003.

"ContraVac: Anatomy of a start-up" Emerging Technology Partners, Rockville, MD Sept. 25, 2003

"Mining the Sperm Proteome for Contraceptive Targets" Indo-US Workshop on Male Contraceptive Research and the Role of Men in Reproductive Health. Oct. 20, 2003, National Institute of Immunology, New Delhi, India.

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome" Center for Cell and Molecular Biology, October 24, 2003 Hyderabad, India.

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome" Center for Research in Reproductive Health, October 27, 2003 Mumbai, India.

"ePAD, an Egg Specific Peptidyl Arginine De-iminase is the Most Abundant Protein in the Ovulated Mammalian Egg and Represents a New Contraceptive Target" Center for Research in Reproductive Health, October 28, 2003 Mumbai, India.

"Patterns of SPAN-X Gene Expression in Human Spermiogenesis, Melanomas and During the Cell Cycle" Laboratory of Biosystems & Cancer, Center for Cancer Research, National Cancer Institute, Bethesda, MD. December 2, 2003

"Rapid Tests for Post Vasectomy Sperm Testing" Expert Consultation on Vasectomy, Family Health International/Engender Health, Washington DC, December 3, 2003

"Opportunities for Collaboration: Acceptability and Utilization of Rapid Tests for Sperm Detection" Joint Maternal and Child Health and Population and Health Centers Network Meeting, NIH Fogarty International Centers, Washington, DC, December 5, 2003

"Update of Human ePAD Intellectual Property Issues" Schering AG, Berlin, Germany December 8, 2003

"Update on Performance of Rapid Tests for Sperm Detection" Schering AG, Berlin, Germany, December 8, 2003

"Patterns of SPAN-X Gene Expression in Human Spermiogenesis, Melanomas and During the Cell Cycle" Schering, AG, Berlin, Germany December 8, 2003

"Intelligent Spermicides: Spermistatic Bivalent Mini-antibodies Targeting Sperm Surface Sugars" Schering AG, Berlin Germany December 9, 2003

"Diagnostic and Therapeutic Products Emerging from the Human Sperm Proteome." Jan. 14, 2004 Repromedix Inc. Boston, Mass.

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome." Dept. of Biomedical Sciences , College of Veterinary Medicine, Cornell University, Ithaca, N.Y. Feb. 3, 2004

"ContraVac: Technology Transfer and Alternative Careers" Johnson School of Management and the Physiology Graduate Student Club, Cornell University, Ithaca, N.Y. Feb. 3, 2004

"ContraVac: Products, People, and Purpose". Piedmont Angel Network, Greensboro, N.C. March 9, 2004

"Mining the Sperm Proteome for Uncharted Regions of of the Human Genome" The Raymond O. Berry Memorial Lecture, Dept. Reproductive Physiology, Texas A&M University, April 2, 2004

"The Technology Underlying Sperm Check" Carrilion Biomedical Institute, Ronoake, VA April 8, 2004

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome" FASEB Symposium Control Mechanisms in Mol. Reproduction, April 21, 2004, Washington, DC

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome". Dept. Pharmacology, Rush University, Medical Center, Chicago, May 12, 2004

"Interdisciplinary Postdoctoral Training in Reproductive Biology and Contraceptive Development for Asian Fellows: The Evolving Experiment". Fogarty International Center, NIH campus, Washington, DC, May 17, 2004.

"Reproductive Biology and Physiology Relevant to Urology". American Urological Association, June 7, 2004. Basic Sciences for Urology residents Course.

"Proteomics and the Discovery of Companion Animal Contraceptive Drug and Vaccine Targets in the Sperm and Egg". Alliance for Contraception in Cats and Dogs, Breckenridge, Colorado. June 27, 2004

"SpermPaints: Fluorescent Monoclonal Antibody Probes for Sperm Identification". 5th Annual DNA Grantees Workshop, National Institute of Justice, Washington, DC, June 29, 2004.

"ContraVac: Anatomy of a Start-up", Center for Innovative Technology, SBIR Phase II Competition, Washington, DC September 15, 2004.

"Proteomics of Isolated Human Sperm Fibrous Sheath: Machinery of Glycolysis and a Novel Sperm Flagellar Energy Carrier Protein" The Future of Male Contraception, Seattle, WA, October 1, 2004.

"SpermCheck® Contraception: An Immunochromatographic Cassette for Monitoring Male Contraceptive Efficacy." Virginia's 10th Annual SBIR Conference, Crystal City, VA, October 13-14, 2004.

"The Role Equatorial Segment Protein in Acrosome Biogenesis" IX International Congress of Reproductive Immunology. Hakone, Japan, October 15, 2004

"SpermPaints and Compliance Testing" Schering Video Conference, December 18, 2004.

"The Role Equatorial Segment Protein in Acrosome Biogenesis." Invited Speaker, Advances and Challenges in Reproductive Health in the PostGenomic Era, Mumbai, India, Jan 13, 2005.

"SpermPaints: Genomics and Proteomics of Sperm Differentiation Biomarkers" Invited Speaker: Bode Technologies Group, Springfield, VA. March 9, 2005

"Equatorial Segment Protein and Acrosome Biogenesis" Invited Speaker: Cell Biology of Fertilization, American Society of Andrology, April 4, 2005.Seattle, WA.

"Reproductive Biology and Physiology Relevant to Urology". American Urological Association, June 6, 2005. Basic Sciences for Urology Residents Course.

"Proteomics of Isolated Human Fibrous Sheath: Machinery of Glycolysis and a Novel Ssperm Flagellar Eneregy Carrier Protein" Invited Speaker: 25th Annual Meeting of the American Society of Reproductive Immunology, Providence, RI, June 17, 2005

"SpermPaints: Fluorescent Monoclonal Antibody Proves to Sperm Differentiation Antigens-Application in Sexual Assault Analysis" Invited Speaker: Sixth Annual DNA Grantees Workshop, US Department of Justice, National Institute of Justice, Washington, DC, June 27, 2005

"Update on the SPAN-XA/D Genes: Role in Human Spermiogenesis" Invited Speaker: National Cancer Institute, Laboratory for Biosystems and Cancer, NIH Campus, Washington, DC, July 28, 2005.

"Fruits of Indo-US Collaboration Between NII-UVA. AIM: To Identify AKAP-3 Interacting Proteins" NIH Indo-US Program on Contraception and Reproductive Health, Rockville, MD. August 22, 2005.

"25 Years After Passage Has the Promise of the Bahy-Dole Act Been Fulfilled?" NIH Indo-US Program on Contraception and Reproductive Health, Rockville, MD. August 24, 2005.

"The SpermPaint Probes for Sexual Assault Analyses" Nassau County District Attorney, Minneola, NY October 10, 2005.

"Analysis of Sexual Assault Smears in the Theresa Fusco Murder", Nassau County District Attorney, Minneola, NY November 1, 2005.

"A Role for the Equatorial Segment in Acrosome Morphogenesis" The Feinstein Institute for Medical Research, North Shore Long Islatnd Jewish Health System, Manhassett, NY November 8, 2005.

Seminars Presented at UVA

"Dissection of the Mouse Sperm Surface with Heterologous Antiserum and Monoclonal Isoantibodies", Internal Medicine, Immunology Conference Group, March 31, 1982.

"Sperm Cell Surfaces," Cell Biology Colloquium, Fall 1981.

"Monoclonal Antibodies to the Mouse Sperm Surface," Immunology Council Faculty Seminar, June 22, 1982.

"Human Hybridomas," Hybridoma Research Group (R.L. Rhebun), November 9, 1983.

"Human Hybridomas Constructed Post Vasectomy," Anatomy - April 1984.

"Monoclonal Antibodies for Use in Analysis of Sexual Assault Evidence". December 5, 1984. Immunology Conference Group.

"Monoclonal Antibodies for Sexual Assault Analysis". Department of Biochemistry, March 21, 1985.

"A Novel Sperm Coating Antigen Originating in the Seminal Vesicle" February 1985. Reproductive Sciences.

"Monoclonal Antibodies: Careers in Biotechnology" April 19, 1985. Biology Association, Gilmer Hall.

"Patenting of Hybridomas for Forensic Markers" November 2, 1985. Board of Directors, University of Virginia Patents Foundation.

"Seminal Vesicle Specific Monoclonal Antibody: A Novel Rape Detection Probe" November 20, 1985. Immunology Conference Group.

"Monoclonal Antibodies in Forensic Detection" September 18, 1987 Clinical Pharmacology/ Toxicology Interdisciplinary Conference; Department of Pathology.

"Monoclonal Antibodies-Strategies and Applications to the Development of a Contraceptive Vaccine" September 22, 1987 Infectious Disease Lecture in Basic Science.

"New Forensic Methods for Identifying Collectable Evidence" October 20, 1987 American Society for Industrial Security (Blue Ridge Chapter).

"A Novel Antigen Within the Human Sperm Acrosome: A Potential Contraceptive Vaccine Immunogen" Dept. Anat. Cell Biol. April 20, 1989.

"Molecular Biology of Human Spermatogenesis" Dept. Rheumatology - June 28, 1989.

"Biochemical and Immunogenicity Studies of a Recombinant Human Sperm Contraceptive Vaccine" December 5, 1990; Immunology Conference Group

"SP-10: A Recombinant Sperm Immunogen in Contraceptive Vaccine Development", Feb. 4, 1992, Department of Pathology

"Restricted Domains of Sperm Antigens Recognized by Monoclonal Antibodies" February 26, 1992, Immunology Conference Group

"Mission of the Contraceptive Vaccine Center" March 28, 1992, University of Virginia Board of Visitors

"Dissecting the Sperm Surface: Applications for Contraception" June 30, 1992, Summer Research Internship Program (MARC/MAAP)

"Sperm Surface Molecules as Contraceptive Vaccine Components" November 17, 1992, Sigma Xi Luncheon Seminar Series, Minor Hall

"Immunologic Aspects and Potentials in Contraception" June 5, 1993, The W. Norman Thornton, Jr. Symposium

"Dissecting the Sperm Surface: Applications for Contraception" July 13, 1993, Summer Research Internship Program (MARC/MAAP)

"Contraceptive Vaccines", July 18, 1994, Summer Research Internship Program (MARC/MAAP)

"Technology Transfer: Are Monoclonal Antibodies to the Sperm Antigen Useful for a Commercial Sperm Diagnostic?" November 20, 1994, Immunology Conference Group

"Steps on the Pathway to Developing a Contraceptive Vaccine", May 15, 1995, American Gynecological Club, Department Ob/Gyn

"Dissecting the Human Sperm Surface: Application for Contraceptive Vaccine Development," June 20, 1995, Medical Academic Advancement Program.

"Keeping It At Home: The Anatomy of a Start Up," Jan. 20, 1996, Faculty Retreat, The Greenbrier, West Virginia.

"Translational Research Panel," Nov. 15, 1996, Dept. Biomedical Engineering

"The Quest for a Contraceptive Vaccine," Jan. 24, 1997, Medical Alumni Council

"The Quest for a Contraceptive Vaccine: Bridging Basic and Applied Science," Feb. 12, 1997, Medical Center Hour

"ContraVac: Anatomy of a Start Up," April 19, 1997, The Acadapreneures Forum, The Darden School

"Strategies in the Development of a Sperm Antigen Based Contraceptive Vaccine," July 16, 1997, Medical Academic Enhancement Program.

"Technology Transfer in Progress at the University of Virginia," October 29, 1997, Dept. Mechanical, Aerospace and Nuclear Engineering.

"Forbidden Knowledge," November 19, 1997, Medical Center Hour.

"Fruits of Leadership in Research- Contraceptive Vaccine Development" Jan. 20, 1998, UVA Medical Alumni Advisory Meeting, Pentagon City, Virginia.

"Translating Basic Discoveries Into Useful Products within Academe" Cardiology Grand Rounds, May 5, 1998

"The Home Sperm Test" SRIP Luncheon, June 19,1998.

"The Quest for a Contraceptive Vaccine" Blue Ridge Chapter of Planned Parenthood. October 12, 1998

"Strategies for Identification of Novel Sperm Surface Proteins" Center for Research in Reproduction Enrichment Program Seminar. December 3, 1998

"The Science and Clinical Uses of Spermcheck: An Immunodiagnostic for Detecting and Measuring Sperm" Molecular Medicine Rounds. May 25,1999

"Oolemma Proteomics: Egg Surface Antigens Important for Fertilization and Contraception" Infectious Disease Faculty Research Forum. September 8, 1999

"Structure of a University Based Biotechnology Company" 2nd Conference on the Development of Technology in Medicine in Virginia, Omni Hotel, Sponsor; Dept. Biomedical Engineering. November 1, 1999

"The Biotechnology Underlying Sperm Immunodiagnostics" Piedmont Virginia Community College's Introduction to Biotechnology Class-Biotechnology Center, 327 W. Main Street, Charlottesville, VA. November 16, 1999, April 5, 2000

"Molecular Events Associated with Human Sperm Capacitation: Oligomerization and Phosphorylation of Calcium Binding Protein 86 [CBP86]" Cell Biology Retreat, September 16, 2000

"Sperm Proteomics" Biotechnology Training Program, October 23, 2000

"Discovery Driven Translational Research in Sperm Proteomics and Genomics: Venturing Beyond the Breakers when the Wind is Strong. Bernie B. Carter Center for Immunology Research, October 25, 2000.

"The Biotechnology Underlying SpermCheck: From Idea to Product" Symposium on Patents and Intellectual Property, Jordan Hall Conference Center, February 6, 2001.

"Sperm Proteomics and the Discovery of Targets for Contraceptive" Wintergreen, Immunology Retreat, June 1, 2001.

"Issues in Intellectual Property Management" Molecular Medicine Round Table, May 1, 2002.

"Innovation and Entrepreneurship" Molecular Medicine Round Table, November 14, 2002.

"Intra-cytoplasmic Sperm Injection [ICSI]: A Grand Experiment in Reverse Eugenics" Critical Human Survival Issue, Foruming Dept. of Anthropology [UNK-45-1]. November 14, 2002.

"Specification of an Equatorial Segment Domain During Early Acrosomal Biogenesis and Insights into the Ancestral Gene Program of Spermiogenesis", Dept. of Biochemistry, November 21, 2002.

"Acadapreneurship: Combining the Academic and the Entrepreneurial, A Case Study in the Transfer of University Intellectual Property to a Biotechnology Start-up." Alternative Careers in Science Symposium, Feb. 26, 2003 sponsored by: The Beirne B. Carter Center for Immunology R, The Cardiovascular Research Center, and the Graduate Programs Office – School of Medicine.

"The Roots of Acadapreneurship: Discovery, Invention, and Innovation" Workshop on Relations with Industry, March 10, 2003, Research Week, Undergraduate Research Network.

"Equatorial Segment Protein and Acrosome Biogenesis" MARC Program, June 26, 2003.

"The Biotechnology Underlying Immunodiagnostics for Sperm." Aug. 4, 2003 Center for Innovative Technology.

"The Biotechnology Underlying Sperm Check®" Sept. 15, 2003 Virginia National Bank.

"Mining the Sperm Proteome for Uncharted Regions of the Human Genome." Sept. 24, 2003 Dept. of Pathology

"Anatomy of a U.VA Biotechnology Start-Up", December 18, 2003. Carillion BioMedical Institute, Site Visit

"SpermPaints for Forensic Science", The John Steve Catilo Memorial Lecture, Univ. of Virginia, October 30, 2004.

"Testis-Specific Biomarkers of Sperm: The Story Behind SpermCheck and SpermPaints" November 6, 2004 83rd Annual Meeting of the Clinical Society of Genitourinary Surgeons, Department of Urology

"SpermPaints: Fluorescent Monoclonal Antibody Probes to Sperm Differentiation Antigens—Applications in Sexual Assault Analysis and Post-Coital Testing" Urology Conference, November 10, 2004.

"Cancer-Testis Antigens, The SPAN-X Gene in Human Spermiogenesis, HPCX, TGCT1, Melanomas and the Cell Cycle." Urology Conference, February 16, 2005.

"Cancer-Testis Antigens, The SPAN-X Gene in Human Spermiogenesis, HPCX, TGCT1, Melanomas and the Cell Cycle." Human Cancer Immunothereapy Conference, Feb 22, 2005

Research Activities Related to University-Industrial Relationships [Translational Research]

Endotronics In 1983 Dr. Herr established a cooperating venture between the University of Virginia and Endotronics, Inc. of Minneapolis, Minn. This resulted in a donation of a $130,000 Acusyst 1501 instrument and a Mini Micro Chamber device to the University. These systems are perfusion devices for cell culture, esp. of hybridomas. The instruments are currently housed in the Chemical Engineering Department.

Flow Labs: Flow Labs donated a Cell Raiser bioreactor ($23,000) to Dr. Herr's lab.

Humagen Fertility Diagnostics: this company, founded by Dr. Herr in 1985, is located in Charlottesville, VA, and manufactures the Penetrak assay, a measure of sperm penetration in cervical mucus for Serono Diagnostics. In addition, the company manufactures pipets for IVF and licenses UVA patents held by the University of Virginia Patents Foundation on forensic assays for rape and blood detection. Dr. Herr worked closely with then Governor Charles Robb's Office of Economic Development to consolidate the venture capital for this enterprise and served as the Chairman of the Humagen Scientific Advisory Board from 1987-1989. In 1996 the company expanded its manufacturing facilities in Charlottesville, Virginia and currently employs~45 persons.

Ortho Pharmaceuticals From 1991-1992 Ortho conducted Contract research within the Center for Recombinant Gamete Contraceptive Vaccinogens. This resulted in direct funding to the Herr lab, costs for animal and primate trials as well as funds for human trials, and royalties to the University.

ContraVac In 1992 Dr. Herr founded ContraVac as a corporate shell to receive technology emanating from the Center for Recombinant Gamete Contraceptive Vaccinogens. ContraVac has licensed several of Dr. Herr's patents and is currently developing a male infertility diagnostic, Sperm Check^{TM} in co-operation with a strategic manufacturing partner, Princeton Bio Medi Tech Corporation. Dr. Herr is working to attract this company to the University North Fork Industrial Park.

Schering, AG In 1998 this company finalized contracts with the University of Virginia and the University of Virginia Patents Foundation to license technology developed in Dr. Herr's and Dr. Flickinger's laboratories. The contracts have resulted in funding for development of young faculty as well as future patent royalties and provides a strategic partnership for translational research.

Graduate Student Thesis Committees Served

Pat E. Bender (L. Rebhun, Major Advisor) Biology (Graduated, 1981)
Fritz Reinhart (R. Bloodgood, Major Advisor) Anatomy
Lynn Samuels (C. Flickinger, Major Advisor) Anatomy (Graduated, 1984)
Wendy Wilson (G. Oliphant, Major Advisor) Anatomy (Graduated, 1986)
David Wolpart (D. Kirwan, Major Advisor) Chem. Engineering
Greg Dandulakis (co-advise with D. Kirwan) Chem. Engineering (Graduated, 1997)
Tina Garza (K. Tung) Pathology (Graduated, 1998)
Bernard Dukes Microbiology (Graduated, 2000)
Tom Gervais (Chemical Engineering/Biotechnology) (Defended July, 2002)
Heping Gheng (Physiology and Molecular Biophysics)
Yan Ge (Rheumatology- Shu Man Fu)

Graduate Student Rotations in Lab

Samuel Waters - CMB - 1994
Elizabeth Norton - Biotechnology - 1994
Connie Grafer - Biotechnology - 1994
Amy Butscher - Biotechnology - 1995
Todd Armstrong - Cell Biology - 1995
Rob Baskin - Molecular Medicine - 1996
Theresa Robinson Thompkins – 2000
Jamie Rolle -2004
Isabel Gonzales-2004

Undergraduate Biology Students Supervised for 495 Projects

1982	Sun Woo Lee
1982	Dana Buchanan
1982	Thomas Gallien
1984	Tracie Rankin
1984	Mike Slattery
1988	Kim Snyder1994-95 Liz Dedman
2001	Maria Chamura
2002	Alexandra Garcia
2003-05	Margaret Samra (won second place in the undergraduate senior thesis competition for her work in the laboratory)

Hughes Scholar Undergraduate Research Program

Brian Kornreich 1990-1992 A Howard Hughes scholar from Biology, Brian worked on the expression of the SP-10 sperm vaccine immunogen. His work won the 1991 Sigma XI Undergraduate Research Award at UVA for his work in the lab.

Medical Student Summer Training (MSTP)

Beverly Boykin	1984	worked on hybridoma production
Tim McGarry	1984	poster on work presented, October 23, 1984, MSTP Poster Session
Andrew Sager	1985	poster on work presented, October 22, 1985, MSTP Poster Session
Tim Polk	1987	Tim worked on immunocytochemistry of the human prostate
Carl Palmer	1989	work on gene cloning from human testis
Ray Dumaran	1989	gene cloning human testis
Samuel M. Brooke	1991	Inhibition of hemi-zona assay by baboon sera to recombinant vaccine.
Renuka Bhattacharya	1994	Monkey oviductal immune response

Medical Minority Academic Advancement Program

1990	Five minority summer students received laboratory experience.
1991	Tounia Louder, a high honor student received training during the summer of 1991.
1996	Joe Boyd (Native American); Jacqueline Barrientos (Hispanic) - spent their summers in the Herr lab.
1997	Glen Davis II (African American) spent the summer in the Herr Lab. Four minority summer students all African Americans, received laboratory experience in the Herr Lab: Fred Cesar, Nieka Harris, Erica Kinney, and Tericka Smith.
1999	Two minority summer students both African Americans, received laboratory experience in the Herr Lab: Gregory Dairyko and Erica Kasper.

2000	Five minority summer students: four African Americans, one Caucasian American, received laboratory experience in the Herr Lab: Janice Hobbs, LaRhonda Jackson, Kwame Osei-Sarfo, Rachel Simpson, and Melissa Stokes.
2001	Six minority summer students: four African Americans, one Caucasian American and one Native American, received laboratory experience in the Herr Lab: Tanisesha Buffin, Ugochi Ekeocha, Eline Haenebalcke, Denise Mayo, Natalie Melrose and Bernadette Ramone.
2002	Five minority summer students: three African Americans, one Caucasian American and one Native American, received laboratory experience in the Herr Lab: Craig Foster, Pierre Gordon, Michelle Morse, Sanet Torres and Nia Washington Plaskett.
2003	Four minority summer students, all four African American, received laboratory experience in the Herr Lab: Kara Malone, Jaime Rolle, Melecia Simpson, Kimberly Wiggins

Activities to Develop Pre-and Postdoctoral Training Resources

Dr. Herr has organized, written, and serves (or served) as program director for several pre- and postdoctoral training programs.

Graduate Training: The Biotechnology Training Grant, a multidisciplinary training program between five basic science departments in the Medical School, Chemistry and the Dept. of Chemical Engineering, was organized and funded by NIH in 1990. Four positions in the first year expanding to six predoctoral positions in the second year were awarded.

Postdoctoral Training: A postdoctoral training program was funded in 1990 within the Center for Recombinant Gamete Contraceptive Vaccinogens which included six postdoctoral positions [five years of funding]. The program will be refunded until 2002.

International Postdoctoral Training: A NIH Fogarty Fellowship Program which funds 4 fellows from India was funded for 5 years beginning in 1995. In 1997 NIH asked if the program would like 2 additional slots and awarded these positions as part of a new initiative in male contraception. In October 2000, the program was competitively renewed for five years with 6 approved positions.

Minority Training: A summer research internship program in reproductive biology for 6 minority undergraduates was funded for 3 years beginning in 1996 by the Mellon Foundation. The program was refunded in 1999 for an additional 3 years.

Activities to Develop New Construction Funds

In 1999 Dr. Herr worked on Capital Hill to improve funding for new construction in the National Center for Research Resources at the NIH. On March 1, 2000 he developed a $2 M grant to NCRR to aid in the construction of the Medical Research-6 Building at the University of Virginia.

11/09/2005

Immunity's pregnant pause

If we can understand why a woman's body does not reject her fetus, it could help us to treat infertility and prevent problems in pregnancy. Helen Pearson reports.

Z. MACAULAY/GETTY IMAGES

BSIP LAURENT/AUBE/SPL

In 1989, a young British woman had her ninth consecutive miscarriage. Her marriage broke down shortly afterwards. But within months of finding a new partner, she had conceived again and the pregnancy went without a hitch. Her daughter is now a healthy and lively nine-year-old.

Reproductive immunologists suspect that the woman's immune system took offence at her first choice of partner — overreacting to tissues carrying his genes and expelling the fetuses he fathered. According to some experts, infertility, recurrent miscarriage, premature delivery and a dangerous complication of pregnancy called pre-eclampsia may all, in some cases, be linked to immunological abnormalities.

If these scientists are right, immunology could be reproductive medicine's next frontier, helping to treat distressing conditions that blight the lives of many couples. "This will be the new area of infertility treatment for this century," predicts Kelton Tremellen of the University of Adelaide in Australia.

In fact, how any fetus survives gestation has baffled scientists for decades: the embryo's tissues are half foreign and yet, unlike a mismatched organ transplant, it isn't normally rejected. Now researchers are revealing how different arms of the mother's immune system react to semen and the implanting embryo — and how the placenta protects itself from her immune system's attack. Some are already building from these findings to develop treatments that could help difficulties in conception and pregnancy. But there is also concern that rogue clinics may exploit preliminary results to offer couples therapies that have yet to be proved safe and effective.

Kelton Tremellen says IVF (left) may be improved by preparing the way with proteins from semen.

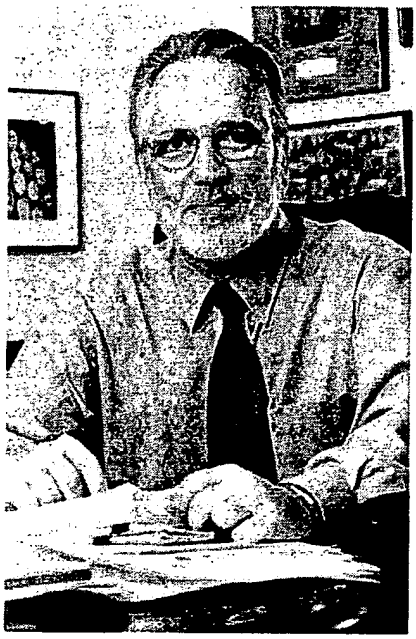
The idea that the immune system may be to blame for problems with pregnancy stems in part from a long-standing observation about pre-eclampsia. This is a life-threatening condition for mother and child affecting up to one in ten pregnancies, in which the placental blood supply is insufficient and can starve a fetus of oxygen. First pregnancies are more susceptible to pre-eclampsia — unless a woman switches partner, in which case a second pregnancy is at equal risk. Previous exposure to a fetus carrying a particular suite of paternal genes, it seems, makes the immune system more likely to tolerate the first-born's subsequent siblings.

More recently, immunologists have wondered whether exposure to proteins in semen helps to prepare a woman's immune system for conception and pregnancy. Tremellen and his colleagues have studied one such protein, called TGF-β, found at high levels in semen. They injected TGF-β into a mouse's uterus alongside a cocktail of foreign proteins, and found that later injections of the same proteins under the skin did not elicit a strong immune reaction[1].

Tremellen, a gynaecologist working in the lab of reproductive immunologist Sarah Robertson at Adelaide, believes that 'immunization' with TGF-β through sexual intercourse helps the maternal immune system learn to tolerate molecular signatures, or antigens, in semen by altering the production of inflammatory molecules called cytokines. He has already shown that *in vitro* fertilization (IVF) is more successful if couples have sex beforehand[2], and hopes soon to begin trials of a vaginal TGF-β gel to see if it can help women suffering recurrent miscarriage.

Although proteins in semen may smooth the way for a future baby, damping down a mother's immune response to the implanting embryo also seems to be crucial to a pregnancy's success. At implantation, a group of embryonic cells called the trophoblast aggressively invades the mother's uterus lining, anchoring the placenta and widening the maternal arteries to enhance its blood supply.

Ashley Moffett-King of the University of Cambridge, UK, and her team have found that natural killer (NK) cells flood the uterus at this time. These immune cells are usually involved in attacking cancerous or virus-infected cells. But those drawn to the uterus



Andrew Mellor (left) and George Chrousos are tracing how a fetus hampers its mother's immune system.

at implantation carry receptors that interact with two unusual antigens, HLA-C and HLA-E, on the surface of trophoblast cells.

Exactly what happens as a result of the interaction between NK cells and the trophoblast is still subject to debate[3]. Moffett-King suspects that it triggers the production of particular cytokines that either help the trophoblast to invade the uterine wall or limit the extent to which it invades. When this process goes wrong, she suggests, the fetal blood supply is compromised and pre-eclampsia might result. If so, a mismatch between HLA antigens on the fetal cells and receptors on the mother's NK cells might predispose certain women to the condition. Moffett-King is now embarking on a study to search for susceptible combinations in 300 British women.

Fetal distraction

Other researchers have discovered several ways in which embryonic tissues disable the mother's immune system — including secreting suppressive factors into her blood or displaying them on placental cells. Another antigen called HLA-G, for example, is released from the trophoblast into the mother's bloodstream, and seems to protect the trophoblast from attack. Researchers led by Philippe Le Bouteiller, who works for INSERM, France's medical research agency, at the Purpan Hospital in Toulouse, have recently shown that soluble HLA-G makes certain types of immune-system T cell — which may attack fetal cells bearing the father's antigens — commit suicide[4]. What's more, a team led by Olavio Baricordi at the University of Ferrara in Italy recently found that implantation after IVF only occurred if the embryos secreted soluble HLA-G (ref. 5).

Based on such findings, Joan Hunt of the University of Kansas Medical Center in Kansas City has already patented a genetically engineered form of human HLA-G. "It could be administered to women with fertility problems," she suggests.

Last year, George Chrousos of the National Institute of Child Health and Human Development in Bethesda, Maryland, presented further evidence that a developing fetus induces some of its mother's T cells to commit suicide. His team showed that corticotropin-releasing hormone (CRH), which is secreted by both the implanting embryo and the lining of the uterus, stimulates trophoblast cells to produce a molecule, known as the Fas ligand, that binds to a cell-surface receptor that triggers cell death. When T cells were grown together with trophoblast cells stimulated by CRH, they died[6].

Later in pregnancy, CRH seems to have an inflammatory role that helps to trigger labour. Chrousos has identified a molecule called antalarmin that blocks the action of CRH (ref. 6), and in unpublished work he found that it seems to delay labour in sheep.

Andrew Mellor of the Medical College of Georgia in Augusta, meanwhile, believes that the placenta cripples maternal T cells in another way: by starving them. In 1998, his team showed that the mouse placenta makes an enzyme called indoleamine 2,3-dioxygenase (IDO), which breaks down an amino acid called tryptophan that is vital for T cells' nutrition. Injections of a molecule that blocks IDO caused high rates of abortion in mice, the team found[7]. "The simple prediction is that problem pregnancies are associated with variations in the IDO gene," says Mellor — although its role in human pregnancy has yet to be demonstrated.

More recently, Mellor's team has shown that the fetal rejection triggered in mice by blocking IDO activates an arm of the immune system called complement[8]. This series of blood plasma proteins is one of the first lines of defence against bacterial and viral infections. But Hector Molina of Washington University in St Louis has shown that it can also hinder pregnancy. Molina's group found that mice lacking Crry, a cell-surface protein that protects tissues against misdirected complement activation, lose their fetuses[9]. Molina now plans to study whether women suffering recurrent miscarriage or pre-term labour have altered levels of proteins that mediate complement, or mutations in the human equivalent of the gene for Crry.

Birth control

With each researcher advocating their favoured mechanism for sustaining gestation, it will be some time before a complete picture emerges. But most agree that mother and fetus use several means in parallel to bring about a peaceful nine months. "There's no definite gene required for gestation," says Mellor. "But if you take one out there's more risk that a pregnancy will be lost."

The eventual goal is to convert the growing knowledge of reproductive immunology into treatments for infertility, miscarriage and other complications of pregnancy. Here, the field's pioneers are battling not only scientific and clinical complexities, but also practitioners operating at the fringes of reproductive medicine who are willing to offer highly speculative 'treatments' to couples desperate to have a baby.

Since the 1980s, for example, women suffering recurrent miscarriage have been offered injections of their partner's white blood cells, supposedly to prime their immune system into accepting a fetus bearing his antigens. Researchers have protested that this is not based on good evidence, and they have been vindicated by a negative randomized clinical trial[10].

Disturbingly, some US doctors are now offering diagnostic tests to determine whether the NK cells in the mother's circulation are abnormal, even though this may be of little relevance to the interactions in the placenta being studied by Moffett-King.

"People try to get the clinical solutions before the science is worked out," says Ian Sargent, an immunologist at the University of Oxford, UK. "It has dogged this area." ■

Helen Pearson works in *Nature*'s news syndication team.

1. Robertson, S. A., Ingman, W. V., O'Leary, S., Sharkey, D. J. & Tremellen, K. P. *J. Reprod. Immunol.* **57**, 109–128 (2002).
2. Tremellen, K. P. *et al. Hum. Reprod.* **15**, 2653–2658 (2000).
3. Moffett-King, A. *Nature Rev. Immunol.* **2**, 656–663 (2002).
4. Fournel, S. *et al. J. Immunol.* **164**, 6100–6104 (2000).
5. Fuzzi, B. *et al. Eur. J. Immunol.* **32**, 311–315 (2002).
6. Makrigiannakis, A. *et al. Nature Immunol.* **2**, 1018–1024 (2001).
7. Munn, D. H. *et al. Science* **281**, 1191–1193 (1998).
8. Mellor, A. L. *et al. Nature Immunol.* **2**, 64–68 (2001).
9. Xu, C. *et al. Science* **287**, 498–501 (2000).
10. Ober, C. *et al. Lancet* **354**, 365–369 (1999).

GENTLE PERSUASION

Plenty of sex before conception could well be the key to encouraging a woman's body to accept a pregnancy. Douglas Fox discovers the unsung virtues of semen

ONE OF THE great mysteries of the human condition is what makes us, unlike most other animals, spend such an inordinate amount of time having sex . . . and so little time actually getting pregnant.

Nor is this a new phenomenon that came along with contraception. Couples still have plenty of sex when conception is out of the question either because of the time of the woman's cycle, or because she is already pregnant or breastfeeding.

But now the mystery of these fruitless bonkings might be solved. According to reproductive biologists at the University of Adelaide in South Australia, far from being an exercise in futility, plenty of sex—even up to a full year before conception—helps guard against a litany of ailments. And Puritans prepare to be shocked—fellatio may work just as well as missionary-style intercourse.

The disorders, which range from infertility to high blood pressure during pregnancy, all appear to be linked to the reluctance of the mother's immune system to accept the fetus and placenta, both of which come armed with an arsenal of foreign proteins courtesy of the father's genes. Sex, early and often, and with the intended father, may help overcome that reluctance, say the Adelaide team.

Clearly, if the mother's immune system remains unconvinced the consequences will be dire. She may immediately and repeatedly reject new embryos—in which case, she's infertile. If her immune system takes a little longer to shun the foreign tissue, she may suffer frequent miscarriages. And if the rejection is milder still it might only affect the placenta—although even that can be a disaster. The placenta is the fetus's lifeline, supplying oxygen and nutrients from the mother's blood. If the placenta fails to grow, or becomes clogged with angry immune cells, the supply line is cut, and an underweight baby is the result or even a stillbirth.

Immune rejection can even threaten the mother's life. According to one still controversial theory, it's the mother's rejection of the placenta that causes pre-eclampsia, a condition where the mother's blood pressure soars, in some cases triggering convulsions, coma and death. In this frightening scenario, fragments of dead placental cells are swept into the mother's circulation, where they damage vessels, sending her blood pressure skywards.

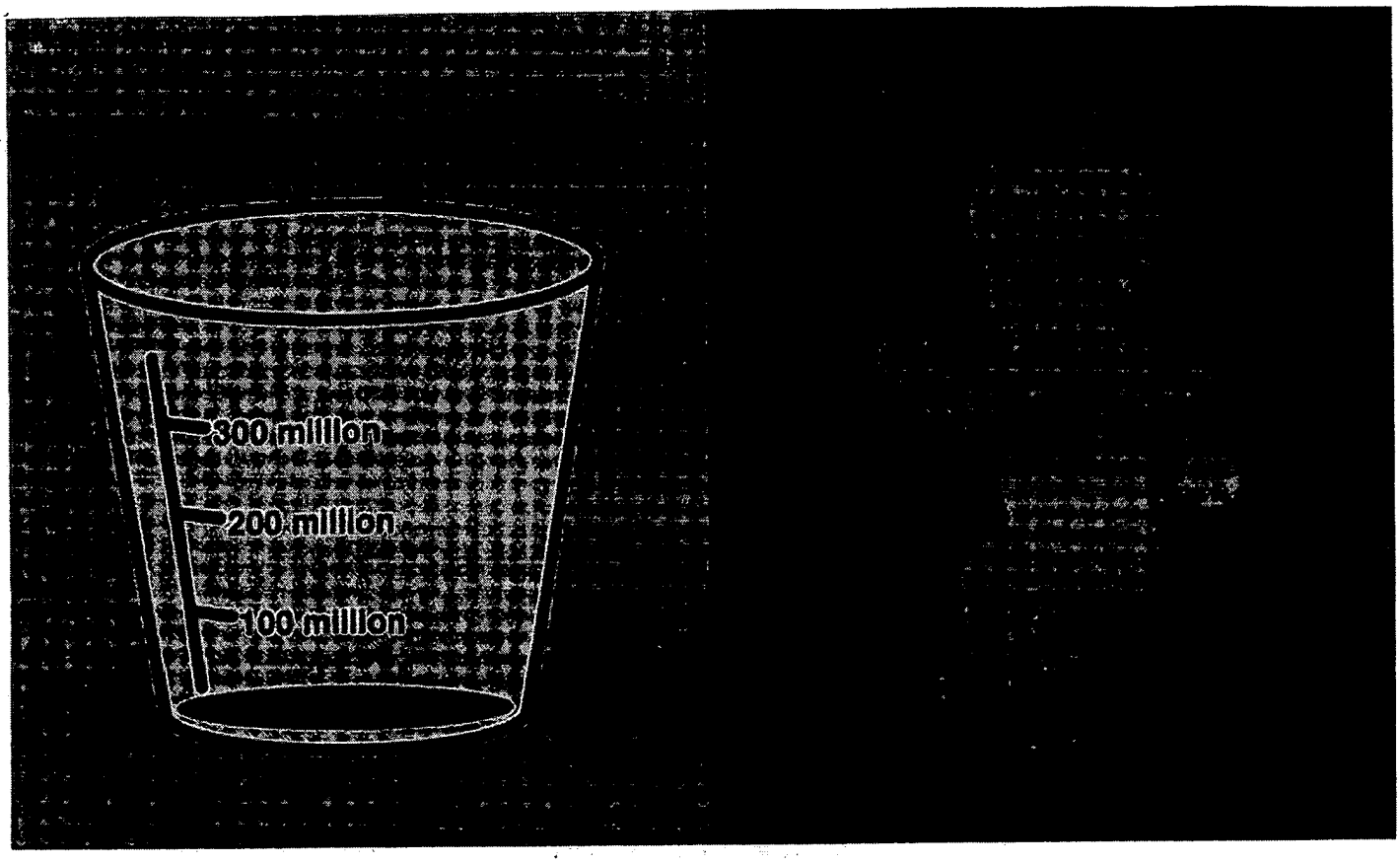
"It's just different expressions of one underlying theme," says Gustaaf Dekker, a member of the Adelaide group. "We see patients that have two miscarriages, then they finally manage to get through their miscarriage period, and they have pre-eclampsia, or the placenta detaches and they have a stillbirth at 24 weeks."

It's easy to understand why the mother's immune system might be tempted to annihilate that developing fetus. As if having foreign genes weren't bad enough, the fetus behaves brutishly during its stay in the womb. Its placenta invades the wall of the uterus like a cancer, infiltrating a nearby artery to guarantee steady supplies of oxygen and nutrients. It also casts millions of foreign cells adrift in the mother's bloodstream, cells that re-attach and grow in places like her lungs. The mother's immune system should positively squash those cells like cockroaches, but amazingly in normal pregnancies it lets them be.

"Acceptance of the conceptus is a much more dynamic affair than anyone's ever given credit for," says Rodney Kelly, a reproductive immunologist at the University of Edinburgh, who strongly suspects that the Adelaide group is on to something. "There's fetal cells in the maternal circulation, there's plenty of antigen, [so] there's obviously an enormous amount of immune modulation preventing rejection."

That process of immune modulation begins with the first drop of semen. You wouldn't suspect it during those dreamy post-coital moments, but for the next 15 hours or so a woman's cervix is swarming with immune cells. They swoop in like government agents investigating an alien crash site—which is essentially what they are doing. They busy themselves collecting the man's foreign proteins—even entire sperm cells—and lug them back to the lymph nodes where other immune cells learn to recognise them. Normally those foreign proteins would end up on the immune system's Most Wanted list: antibodies would be made against them, and primed to annihilate the sperm next time they dared to


C-men
For the prevention of infertility, miscarriage, high blood pressure during pregnancy, stillbirth and underweight babies
Take frequently for at least 1 year
C-men is not intended for use with a condom
INGREDIENTS:
Foreign proteins
Immune modulators
Prostaglandins
TGF-beta
Contains 100 million sperm per dose
WARNING:
DO EXCEED STATED DOSE
100 million: 01



darken their doorstep. But the miracle of sex is that semen contains not only millions of sperm loaded with foreign proteins, but also some recently discovered components that tilt a woman's immune response away from hostility and toward acceptance.

"If there's repeated exposure to that signal," says Dekker, "then eventually when the woman conceives, her [immune] cells will say, 'we know that guy, he's been around a long time, we'll allow the pregnancy to continue.'"

Of course acceptance of the sperm by the mother's immune system isn't all that's needed for a straightforward pregnancy. But when things do go wrong, sperm have the power to provoke a vicious immune response. Following intercourse, women very occasionally go into anaphylactic shock, an immune response so severe that breathing can be nigh on impossible, and blood pressure plummets dangerously low. For these luckless women, just a drop of semen on a thigh can raise boils. The problem appears to stem from lacklustre attempts by the woman's immune system to become tolerant to sperm, combined with the man's semen doing a really bad job of convincing it. In some cases switching partners is all it takes to solve the problem.

And there's other tantalising evidence of semen's power over the mother's immune response. An analysis of pre-eclampsia patterns in 1.7 million births from the Medical Birth Registry of Norway found that certain "dangerous males" are nearly twice as likely to father a pre-eclamptic pregnancy. These dangerous males carry their high risk from one female partner to the next.

But these are the rare examples where a man's semen isn't functioning properly. It was Pierre-Yves Robillard, a neonatologist now at the Sud Réunion Hospital on Réunion in the Indian Ocean, who showed that under normal circumstances semen exposure actually helps prevent pre-eclampsia—evidence that flies in the face of mainstream explanations for the disorder such as the popular "pantyhose" theory.

According to this theory, pre-eclampsia develops not because the mother's immune system sees the placenta as foreign, but because the blood vessels that supply the placenta don't expand enough. The placenta runs short of oxygen, and once again dying cells push up the woman's blood pressure. By a second pregnancy the blood vessels are already widened like worn pantyhose, which is why, say the textbooks, pre-eclampsia usually only happens in a first pregnancy. According to Robillard's studies, however, later pregnancies can be just as risky under certain circumstances.

In the late 1980's, Robillard was on the French island of Guadeloupe in the Caribbean, a territory dotted with sugar and banana plantations, where families are often made up of a single woman who has several children by different men. There he made the curious discovery that most of his patients with pre-eclampsia were actually on their second or third pregnancy. But it was specifically the women who had changed partners since their last pregnancy who were developing the condition. Robillard speculated that the mother's immune system requires time (and contact with semen) to learn to accept the father's foreign genes and not attack the placenta and cause pre-eclampsia. Changing fathers between pregnancies "puts your counter back at zero immunologically speaking", he says.

He confirmed his hunch in his next study of 1011 pregnant women on Guadeloupe. Women who had sex with the father for 12 months or more before getting pregnant had a 5 per cent chance of developing pre-eclampsia compared to a massive 40 per cent chance for those who'd only been having sex with the father for four months or less. What's more, another study found that using condoms, which naturally prevent women from coming into contact with semen, increases the risk of pre-eclampsia.

Robillard's studies make an excellent case for the medicinal virtues of semen exposure. But it was Dekker, then at the Free University of Amsterdam, who took the studies one eyebrow-raising step further when he looked to see if the same goal could be achieved with oral sex—or more specifically, fellatio.

It's well known that our immune systems tolerate things better when they enter the

body via the mouth. This is why we're not usually allergic to our food even though it's always genetically foreign, and why girls with nickel braces on their teeth are less likely to develop nickel allergies after their ears are pierced than girls without these braces.

Sure enough, when Dekker compared 41 pregnant women with pre-eclampsia and 44 without, he found that 82 per cent of those without pre-eclampsia practised fellatio, compared with only 44 per cent of those with the disorder. And in keeping with the "condom effect", the protective effect of oral sex was strongest if the woman actually swallowed the semen rather than coughing it onto the pillow. True, it's only one study, but for some couples who can't seem to carry a pregnancy to term, a little fellatio can hardly do any harm, suggests Dekker. "I tell them, 'semen exposure's good, and you could think of oral sex.'"

Some people are a little less gung-ho. "The idea is cool," says James Roberts, director of the Magee-Women's Research Institute at the University of Pittsburgh, "but generating data that's not confounded is very difficult because sexual practices aren't independent of one another." The couples indulging in oral sex might, for example, have more sex overall.

Sure they could, acknowledges Dekker, "but even then it still supports the same message—that semen exposure is protective."

Roberts also points out a potential weakness in Robillard's father-switching data from Guadeloupe: it's possible that pregnancies with new partners tend to happen longer after the last pregnancy than those with the same father, which might mean that increased pre-eclampsia is triggered not by lack of exposure to semen, but by the stress of re-expanding uterine blood vessels that have shrunk back down since the last pregnancy. Still, this is just a minor quibble. "There are other ways to read the data," says Roberts, but there's nothing that holds together quite as well as the idea of immune rejection contributing to pre-eclampsia, and semen exposure preventing it.

If you are wondering whether the exhortation for fellatio is a case of male fantasies hijacking science, as one *New Scientist* editor thought, consider that the Adelaide group is spearheaded by a woman—reproductive biologist Sarah Robertson.

Robertson and obstetrician Kelton Tremellen have already helped show that one component of semen (see below) plays a key role in persuading the mother's immune system to accept foreign sperm and a foreign fetus—a discovery that could lead to medical treatments that are more refined than your basic fellatio. "We might be able to devise artificial therapies to augment natural intercourse or maybe even replace natural intercourse in people who have problems getting this immune thing going on their own," says Robertson.

Their key component is called transforming growth factor beta. TGF-beta summons immune cells to the woman's cervix after sex to gather the man's foreign proteins. And according to Robertson and Tremellen's mouse studies, TGF-beta also acts as a switch, transforming what would usually be a hostile reaction to sperm from the immune cells into a friendly one. When the two researchers injected sperm protein into mouse uteruses, then injected the same protein under the skin of the mice a few days later, it triggered a severe allergic reaction—unless the first injection also included TGF-beta.

What makes their discovery particularly exciting as a potential therapy is that TGF-beta has this effect the first time sperm enter the vagina, although, says Robertson, repeated exposure to the sperm and TGF-beta is probably necessary for complete tolerance.

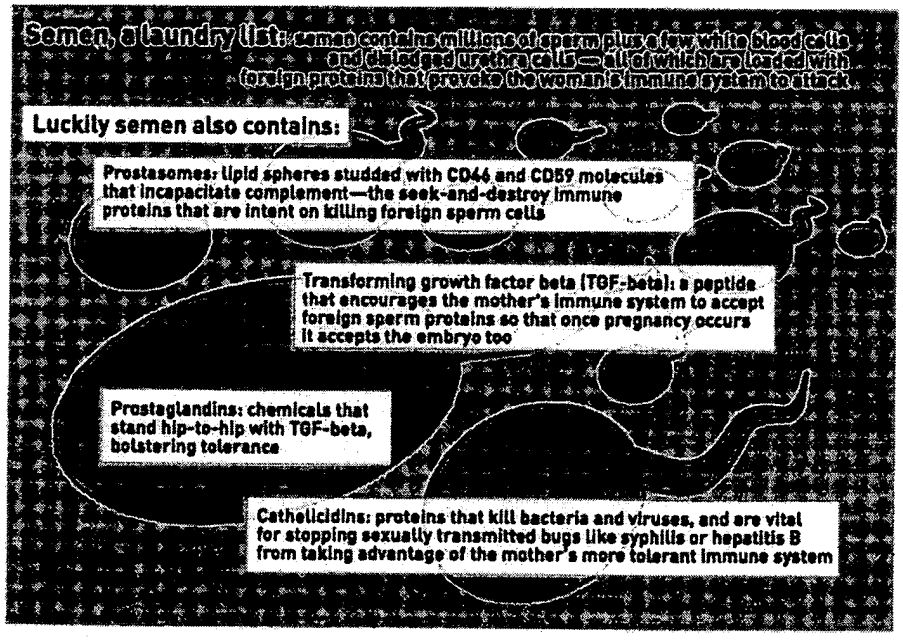
'THEIR HUNCH IS THAT DANGEROUS MEN DON'T SPORT ENOUGH TGF-BETA'

Dekker and Tremellen are currently comparing TGF-beta levels in the semen of men who have fathered normal pregnancies with the dangerous males where conception has ended in miscarriages or pre-eclampsia. Their hunch is that dangerous males simply don't sport enough TGF-beta in their semen. If they are right, the next step will be to treat women who suffer repeated miscarriages or IVF failures with TGF-beta.

Of course, the TGF-beta will have to be given along with the father's foreign proteins, which means during intercourse, perhaps in a vaginal gel. Intercourse during an IVF cycle is already known to up the chances of pregnancy (*New Scientist*, 9 December 2000, p 6). Tremellen suspects that's partly due to the TGF-beta in the semen. The gel, he says, would provide an additional boost.

Nor are the potential pay-offs to understanding how a woman's immune system tolerates a fetus for nine months confined to reproductive medicine. Autoimmune diseases such as lupus and multiple sclerosis, where the body's immune system attacks its own organs, are another target. "There's nothing to say we couldn't deliver myelin in a vaginal immunisation that might benefit women with MS," says Tremellen.

The Adelaide group's work is also satisfying for other reasons more to do with, eh, lifestyle. After all, it provides validation for what many people already hoped—that all those long, lingering Saturday mornings in the sack are anything but a wasted effort. □



Douglas Fox is a science writer living in northern California

Further reading: "The role of semen in induction of maternal immune tolerance to pregnancy" by Sarah Robertson and David Sharkey, *Seminars in Immunology*, vol 13, p 243 (2001)
"Correlation between oral sex and a low incidence of pre-eclampsia: a role for soluble HLA in seminal fluid?" by Carin Koelman and others, *Journal of Reproductive Immunology*, vol 46, p 155 (2000)

EXHIBIT 1

Recombinant Latent Transforming Growth Factor β1 Has a Longer Plasma Half-Life in Rats than Active Transforming Growth Factor β1, and a Different Tissue Distribution

Lalage M. Wakefield,* Thomas S. Winokur, Robin S. Hollands, Karen Christopherson,‡ Arthur D. Levinson,‖ and Michael B. Sporn
Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892; and the Department of Cell Genetics, Genentech Inc., South San Francisco, California 94080

Abstract

Transforming growth factor β1 (TGF-β1) is a key regulator of cell growth and differentiation. Under normal physiological conditions, it is made as a biologically latent complex whose significance is unknown. Previous work has indicated that active TGF-β1 has a very short plasma half-life in rats (Coffey, R. J., L. J. Kost, R. M. Lyons, H. L. Moses, and N. F. LaRusso. 1987. *J. Clin. Invest.* 80:750–757). We have investigated the possibility that latent complex formation may extend the plasma half-life of TGF-β1 and alter its organ distribution. Radiolabeled latent TGF-β1 was formed by noncovalent association of ^{125}I-TGF-β1 with the TGF-β1 precursor "pro" region from recombinant sources. TGF-β1 in this latent complex had a greatly extended plasma half-life (> 100 min) in rats compared with active TGF-β1 (2–3 min). Whereas active TGF-β1 was rapidly taken up by the liver, kidneys, lungs, and spleen and degraded, TGF-β1 in the latent complex was largely confined to the circulation, and was < 5% degraded after 90 min. The pharmacokinetics of TGF-β1 in the latent complex were shown to be critically dependent on the degree of sialylation of the complex. The results suggest that formation of latent complexes may switch endogenous TGF-β1 from an autocrine/paracrine mode of action to a more endocrine mode involving target organs distant from the site of synthesis. (*J. Clin. Invest.* 1990. 86:1976–1984.) Key words: transforming growth factor β1 • latent complex formation • plasma half-life

Introduction

Transforming growth factor-β1 (TGF-β1)[1] is the prototype of a family of proteins that is intimately involved in the regulation of virtually every physiological process (for review see reference 1). Experiments in animal model systems have indicated that TGF-β1 has great clinical potential in the areas of wound healing (2), immunosuppression and myeloprotection (3), and tumor ablation (4). Human clinical trials for TGF-β1 in some of these areas are anticipated in the coming 5 yr.

TGF-β1 differs from the majority of growth regulatory factors in that it is generally synthesized and secreted in a biologically latent form, and this must be activated before TGF-β1 can exert its biological effects on target cells (5, 6). The nature of the activation mechanism in vivo is unclear, but may involve proteases, and in some instances may be dependent on cell–cell interactions (7–9). The major latent form of TGF-β1 that occurs naturally is a high molecular weight complex, in which the homodimeric active TGF-β1 is noncovalently associated with a dimer of the remainder of its precursor "pro" region, and this in turn is disulfide-bonded to a third, structurally unrelated protein of 135 kD (10, 11).

Recombinant systems expressing the entire coding region of the TGF-β1 gene also make TGF-β1 in a latent form (12, 13). However, in this case the latent complex consists only of active TGF-β1, noncovalently associated with the precursor "pro" region. This indicates that the precursor "pro" sequence alone is sufficient to confer latency on TGF-β1, and the 135-kD protein of the natural latent complex must have a different function. The dimeric form of the TGF-β1 precursor "pro" region is now termed the TGF-β1 latency-associated peptide (LAP).

The in vivo significance of the latent form of TGF-β1 is unclear. However, since it appears to be the predominant naturally occurring form of the molecule, it may be a more appropriate form of TGF-β1 to use in many clinical settings than the active molecule. Given the pleiotropic effects of active TGF-β1 on virtually every organ system, systemic administration of active TGF-β1 may bypass normal regulatory mechanisms that operate on latent TGF-β1 to restrict its activity spatially and temporally, and could result in undesirable responses in non-target organs. Furthermore, recent data have indicated that active TGF-β1 has an extremely short plasma half-life in rats (14). In the present work we have asked whether one role of latent complex formation might be to extend the plasma half-life of TGF-β1, and to alter its organ distribution. To do this, we have compared in rats the relative pharmacokinetics of active TGF-β1, and latent TGF-β1 complexes, formed by recombining ^{125}I-TGF-β1 with TGF-β1 LAP from recombinant sources.

Methods

Assays for TGF-β1 and LAP

Radioreceptor assays. Porcine platelet TGF-β1 was obtained from R&D Systems Inc. (Minneapolis, MN), and iodinated to a specific activity of ~ 80 μCi/μg by the modified chloramine T method (15). The latent TGF-β1 complex was quantitated by radioreceptor assay

Address correspondence to Dr. Lalage M. Wakefield, Laboratory of Chemoprevention, Building 41, Room B1111, National Cancer Institute, Bethesda, MD 20892.

Received for publication 9 March 1990 and in revised form 20 July 1990.

1. *Abbreviations used in this paper:* α_2M, α_1-macroglobulin; BS^3, bis-sulfosuccinimidyl suberate; DHFR, dihydrofolate reductase; LAP, latency-associated peptide; MTX, methotrexate; TGF-β1, transforming growth factor β1; S-LAP, sialylated LAP; U-LAP, undersialylated LAP.

(13). The LAP was assayed by its ability to inhibit the binding of ^{125}I–TGF-β1 to its receptor on A549 cells after preincubation of samples with ^{125}I]-TGF-β1 for 2 h at 20°C to allow complex formation to occur. A unit of LAP activity was defined as the amount of LAP required to cause 50% reversal of the specific binding of 250 pg ^{125}I–TGF-β1 to receptors on A549 cells. 40 U LAP was sufficient to neutralize completely 1 ng TGF-β1.

Native agarose gel electrophoresis. Latent complexes formed between ^{125}I–TGF-β1 and LAP proteins were visualized by native agarose electrophoresis in uncharged, 0.5% composite agarose-galactomannan gels (Isogel; FMC Bioproducts Corp., Rockland, ME) essentially according to McCaffrey et al. (16). Gels were prepared with 50 mM Tris-acetate buffer, pH 7.0, and run at 100 V for ~ 6 h with cooling. Dried gels were exposed overnight to G-Mat film (Eastman Kodak Co., Rochester, NY).

Chemical cross-linking and SDS-PAGE analysis. Latent complex formation was also analyzed by denaturing gel electrophoresis under nonreducing conditions, using 3–15% polyacrylamide gradient gels. The noncovalent latent complex was stabilized before electrophoresis by chemical cross-linking of samples with 2 mM bis-sulfosuccinimidyl suberate (BS^3; Pierce Chemical Co., Rockford, IL) for 30 min at 4°C. In the absence of cross-linking, the noncovalent complex is dissociated by the SDS in the electrophoresis sample buffer. Cross-linking analysis allows the molecular weight of the latent complex to be determined. However, since the cross-linking reaction is relatively low efficiency (~ 25%), it does not indicate what fraction of total TGF-β is in the latent form. Dried gels were exposed to Kodak X-Mat film for 36 h to visualize bands.

Transfections and selections

A vector encoding TGF-β1 (pSBβ, reference 13) was cotransfected into dihydrofolate reductase–negative Chinese hamster ovary (DHFR$^-$ CHO) cells (17) with the DHFR vector pFD11 (18). Cells expressing DHFR were selected as described (18). DHFR$^+$ cells were screened for TGF-β1 expression by metabolic labeling and RIA (13). Cells positive for TGF-β1 expression were treated with increasing concentrations of methotrexate (MTX) to select derivatives having amplified copies of the transfected plasmids. Two clones exhibiting elevated levels of TGF-β1 expression were subjected to further analysis: clone MJβ1, selected in 100 nM MTX; and clone C3/12A, selected in 1 μM MTX. Subsequent analysis (see Results) revealed that clone MJβ1 represented a variant CHO cell (19) that produced a form of latent TGF-β1 deficient in sialic acid.

Medium collection

Cells were seeded into eight 2-liter roller bottles at a density of 3.3 × 10^5 cells per bottle. The medium was a modified mixture of selective Ham's F-12 and DME with additions including either 100 nM (clone MJβ1) or 1 μM (clone 3/12A) MTX and 2% extensively diafiltered serum. After 5 d, the cells were grown in a serum-free mixture of Ham's F-12 and DME. Cell supernatants were harvested after 7 d and concentrated from an initial volume of 2.5 liters to ~ 200 ml by ultrafiltration in a Stir Cell (Amicon Corp., Danvers, MA) (30-kD cutoff).

Purification of LAP

All initial clearance experiments were performed on LAP purified from the MJβ1 clone. The conditioned medium was dialyzed against 20 mM Tris-Cl, pH 7.5, and fractionated on a Mono Q HR16/10 anion-exchange column. All chromatography steps were performed at room temperature using a fast protein liquid chromatography system (Pharmacia-LKB Biotechnologies Inc., Piscataway, NJ.) The latent TGF-β1 complex eluted in the flow-through, and was then dialyzed against 20 mM sodium phosphate, pH 7.0, containing 8 M urea to dissociate the latent complex. The dialyzed material was fractionated on a Mono S HR5/5 cation exchange column, developed with a 70 ml gradient of 0–0.3 M NaCl, in the presence of 8 M urea. The TGF-β1 LAP eluted at 0.08–0.09 M NaCl, whereas free TGF-β1 eluted at 0.13 M NaCl. Pooled peak fractions were concentrated by ultrafiltration using a Centricon 10 device, and further purified by gel filtration on a Superose 12 column with PBS containing 10% glycerol as the elution buffer. The LAP eluted with a retention volume of 14 ml. The final material was 80–90% pure as judged by SDS polyacrylamide gel electrophoresis. Since subsequent analysis (see Results) revealed this material was undersialylated, it is referred to as U-LAP.

For purification of TGF-β1 LAP from the C3/12A clone, the conditioned medium was dialyzed and loaded on a Mono Q anion-exchange column as above. Since the latent complex produced by this culture system was anionic, due to more extensive sialylation of the complex than occurred with the MJβ1 clone (see Results), it bound to the column and was eluted at 0.05–0.1 M NaCl. Pooled peak fractions were dialyzed against 20 mM Tris-Cl, pH 7.5, containing 6 M urea, and fractionated on a Mono Q HR5/5 column, using a 0–0.3 M NaCl gradient in the presence of 6 M urea. The LAP eluted at 0.11–0.14 M NaCl, well separated from free TGF-β1 that eluted in the flow through, and from the bulk of contaminating protein that eluted at lower ionic strength. Pooled peak fractions were further purified on Superose 12 as above. SDS-PAGE analysis indicated the LAP was 40–50% pure. Because of the more extensive sialylation of this species, this material is referred to as S-LAP.

Neuraminidase digestion

The sialic acid content of the LAP samples was examined by comparing the molecular weight of the reduced LAP species on Western blots, before and after digestion of conditioned media or partially purified LAP protein overnight at 37°C with 14 U/ml soluble or immobilized neuraminidase (Type XA; Sigma Chemical Co., St. Louis, MO) in 100 mM sodium acetate, pH 5.6, containing 1 μg/ml leupeptin and pepstatin, and 2 μg/ml aprotinin. Immunoblots of the conditioned media samples were probed with a polyclonal antibody against a synthetic peptide corresponding to residues 46–56 of the TGF-β1 precursor to visualize the LAP, as described (13).

Pharmacokinetic studies

Labeled latent complex for the pharmacokinetic studies was made by incubating ^{125}I–TGF-β1 with an excess of LAP (≥ 40 U LAP/ng TGF-β1), in PBS, overnight at 4°C. Each animal received the equivalent of 20–160 ng ^{125}I–TGF-β1, corresponding to 1–8 μCi, in a volume of 0.5 ml. No difference in tissue distribution or plasma half-life was observed over this dose range. All experiments were conducted with male Sprague-Dawley rats weighing between 225 and 295 g. Rats were anesthetized with intraperitoneal pentobarbital (50 mg/kg). A PE10 catheter was introduced into the right iliac artery, and animals were heparinized by injection of ~ 45 U of heparin in 0.1 ml PBS. Samples were injected into the contralateral femoral vein in a total volume of 0.5 ml. Blood samples of 0.15–0.2 ml were withdrawn from the iliac artery at timed intervals after sample injection. 10 U heparin was immediately added and plasma was prepared by spinning out the blood cells for 2 min in a microfuge. Both plasma and blood cell fractions were counted for radioactivity. Essentially no radioactivity was recovered in the blood cell fraction (data not shown). The extent of degradation of the labeled material in the plasma was assessed by determining the fraction of label that was precipitable by 20% TCA. Plasma half-lives for the various labeled species were determined from the rate of decay of TCA precipitable counts in the plasma with time. The distribution volumes were determined by extrapolation of plasma radioactivity to $t = 0$ min, using the initial linear portion of the decay curve on a log plot. At the end of the time course, the animals were killed by pentobarbital overdose and organs were excised, weighed; and counted for ^{125}I in a gamma counter. In some experiments the organs were then fixed in neutral buffered formalin for autoradiography. In others, the radioactive material in the organs was recovered by acid-ethanol extraction. Briefly, organs were minced, and 4 ml/g tissue of an ice-cold solution consisting of 93% EtOH, 230 mM HCl, 1 μg/ml PMSF, and 5 μg/ml pepstatin was added. The tissue was immediately homogenized in a tissuemizer. Homogenates were then rocked over-

night at 4°C and centrifuged for 30 min at 3,000 *g* to remove insoluble material. The extent of degradation of the labeled material in each organ was then assessed by determining the fraction of extracted ^{125}I that was precipitable by 20% TCA.

Since heparin has been shown to affect the association of active TGF-β and α-2-macroglobulin in vitro (16), one experiment was performed in which the clearance of active TGF-β was monitored in an unheparinized animal. Although blood clotting in the catheter prevented the construction of a complete plasma clearance curve, the time points that were obtained were essentially superimposable on those obtained from heparinized animals, and the distribution of label between organs at the end of the experiment was identical in heparinized and nonheparinized animals (data not shown). All animals thereafter were heparinized.

Results

Formation of iodinated latent TGF-β1 complexes. Iodinated latent TGF-β1 complexes were formed by recombining partially purified recombinant LAP species with ^{125}I-TGF-β1 of high purity. This approach was chosen since TGF-β1 is relatively inaccessible in the latent complex and iodination of intact latent complex would be expected to label the LAP rather than the TGF-β1 (13). All recombinant LAP species caused a dose-dependent inhibition of the ability of ^{125}I-TGF-β1 to bind to its receptor on A549 cells, indicating that latent complexes had formed (Fig. 1). Treatments that are known to activate latent TGF-β1 complexes, such as transient acidification to pH 3, or heating to 80°C for 5 min (20), restored the ability of the ^{125}I-TGF-β1 to bind to its receptor, indicating that the reformed latent complex can be activated in the same way as native complexes. Agarose gel electrophoresis under nondenaturing conditions allowed visualization of complex formation. Iodinated TGF-β1 alone did not migrate from the origin under these conditions (Fig. 2 *A*, lanes *1* and *2*). However, when combined with U-LAP, the form of LAP made by the MJβ1 clone, a new species formed that migrated towards the cathode (Fig. 2 *A*, lane *3*). Heating the sample to 80°C for 5 min resulted in the disappearance of this species (Fig. 2 *A*, lane *4*). Similar results were obtained with S-LAP, the form of LAP that was made by the C3/12A clone. However, in this case the complex had a net negative charge and migrated in the opposite direction (Fig. 2 *A*, lanes *5* and *6*). Taken together, the data indicate that the partially purified LAP species are capable of recombining with iodinated TGF-β1 to form biologically latent complexes.

Pharmacokinetics of latent TGF-β1 in rat. The latent TGF-β1 complexes were injected into rats to determine the plasma half-lives, and the results are presented in Table I. Active TGF-β1 was cleared very rapidly from rat plasma with a half-life of 2–3 min, in agreement with previously published work (14). Initial experiments in which latent TGF-β1 was

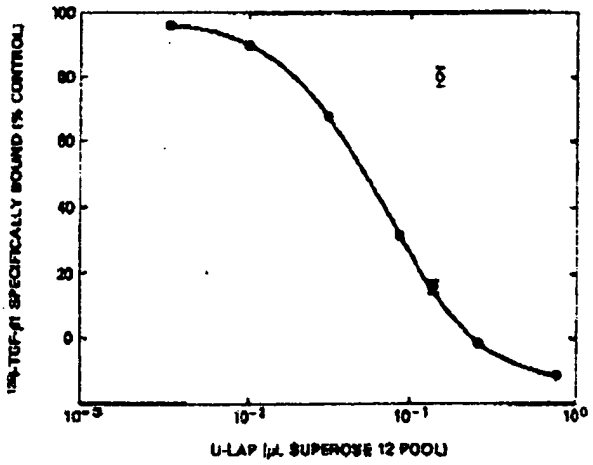


Figure 1. Inhibition of binding of ^{125}I-TGF-β1 to cell surface receptors due to complex formation with LAP species. Varying concentrations of U-LAP were preincubated with 50 pM ^{125}I-TGF-β1 to allow complex formation to occur, and uncomplexed TGF-β was assayed by its ability to bind to TGF-β receptors on A549 cells, as described in Methods. The sample volume/well was 200 μl, corresponding to 250 pg TGF-β, and control samples had no added LAP. In the absence of added LAP, total binding was 3,395±39 cpm/well, and the nonspecific binding, determined in the presence of 10 nM unlabeled TGF-β, was 489±49. A unit of LAP activity was defined as that amount of LAP required to give 50% inhibition of the specific binding of ^{125}I-TGF-β1 in this assay system. At high concentrations, LAP reversed nonspecific binding as well as specific binding of ^{125}I-TGF-β1. The open circle indicates binding observed when the U-LAP/^{125}I-TGF-β1 complex is activated by heating at 80°C for 5 min. Essentially identical results were obtained with preparations of S-LAP (not shown).

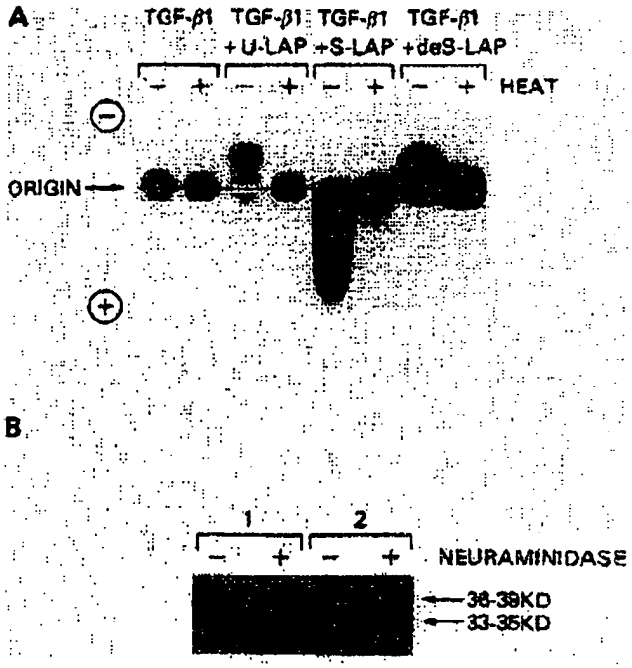


Figure 2. (*A*) Native agarose gels of recombinant latent TGF-β1 complexes. Complexes were formed between ^{125}I-TGF-β1 and recombinant LAP species, and analyzed on agarose gels, with and without activation of the complex, by heating to 80°C for 5 min, as described in Methods. U-LAP, LAP from the MJβ1 clone; S-LAP, LAP from the C3/12A clone; deS-LAP: S-LAP desialylated with neuraminidase. (*B*) Immunoblot analysis of extent of sialylation of LAP. LAP species in serum-free conditioned medium from C3/12A clone (sample *1*: lanes 1 and 2) or MJβ1 clone (sample *2*: lanes 3 and 4) were compared in immunoblots before and after removal of sialic acid with neuraminidase as described in Methods.

Table I. Effect of Latent Complex Formation on Plasma Half-Life and Distribution Volume of TGF-β1 in Rat

Form of TGF-β	Plasma $T_{1/2}$	V_d
	min	*ml*
Active TGF-β1		
TGF-β1	2.7±0.4	81.5±26.5
Latent TGF-β1		
TGF-β + U-LAP	1.2±0.3	40.0±5.6
TGF-β1 + U-LAP + asialofetuin	9.2±1.4	15.2±2.7
TGF-β1 + S-LAP	108.6±8.2	17.1±2.3
TGF-β1 + deS-LAP	1.2	27.4

^{125}I-TGF-β1 was incorporated into a latent complex with different forms of LAP, and injected into the femoral veins of rats. The plasma half-lives were determined from the decrease in TCA-precipitable radioactivity in the plasma with time. For active TGF-β1, and latent complexes formed with U-LAP and deS-LAP, the decrease in radioactivity was biphasic and half-life determinations were made for the initial rapid phase, only using time points up to 5 min after injection. The distribution volumes were determined by extrapolation to t = 0 of the initial linear portion of the decay curve in a log plot. Data are the mean±SD of three determinations, except for TGF-β1 alone (n = 6) and TGF-β1 + deS-LAP (n = 1).

formed from ^{125}I-TGF-β1 and U-LAP gave the unexpected result that the latent complex was cleared even more rapidly than the active TGF-β1. Since the labeled material went almost exclusively to the liver (data not shown), and the latent complex was cationic, we reasoned that the complex might be undersialylated and be cleared through the asialoglycoprotein receptor in the liver. This receptor is known to scavenge aged desialylated plasma proteins (21). Co-injection of an excess of asialofetuin to saturate the asialoglycoprotein receptor resulted in a greater than sevenfold increase in the half-life of the latent complex, consistent with its clearance via this route (Table I). Western blot comparison of the LAP protein in conditioned media from the MJβ1 and C3/12A clones, before and after treatment with neuraminidase, indicated that the LAP made by the MJβ1 clone (U-LAP) was undersialylated compared with that from the C3/12A clone (S-LAP) (Fig. 2 *B*). The molecular weight of the reduced S-LAP (36–39 kD) was similar to that observed for LAP derived from human platelet latent TGF-β (10). Latent complex formed with S-LAP had a greatly extended plasma half-life of > 100 min (Table I). Removal of sialic acid from S-LAP by neuraminidase digestion resulted in formation of a latent complex with the same very short half-life as complexes formed with U-LAP. Representative plasma decay curves for ^{125}I-TGF-β1 with and without S-LAP are shown in Fig. 3.

Tissue distribution of latent and active TGF-β1. The distribution volume (V_d) of active TGF-β1 was ~ 80 ml, suggesting that active TGF-β1 leaves the circulation and distributes into

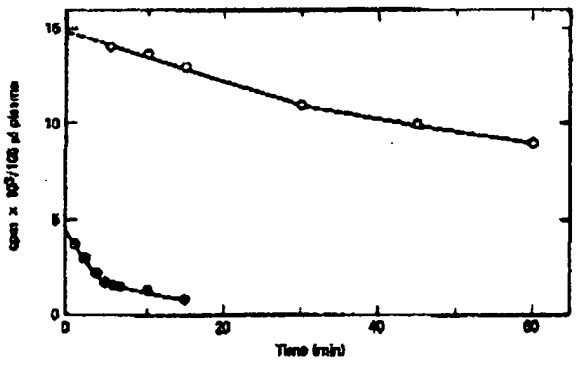


Figure 3. Effect of latent complex formation on the disappearance of circulating ^{125}I-TGF-β1 from rat plasma. ^{125}I-TGF-β1, alone or complexed with S-LAP, was injected into the femoral artery and the TCA precipitable counts in the plasma were determined at various times after injection, as described in Methods. Data points are the TCA precipitable cpm in 100 μl plasma, normalized to 5 × 10^4 cpm injected. In this experiment, the plasma half-life for the active TGF-β was 3.1 min, and that for the latent TGF-β was 110.0 min. Data collection for the latent complex was continued up to t = 90 min to allow more accurate determination of the half-life. Active TGF-β (●), latent TGF-β (TGF-β + S-LAP) (○).

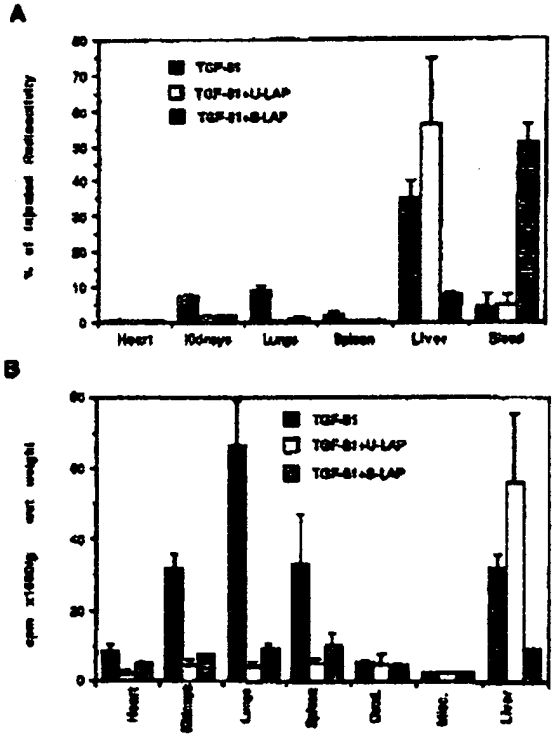


Figure 4. Tissue distribution of radioactivity after intrafemoral administration of ^{125}I-TGF-β1 with or without added LAP to form latent TGF-β1 complexes. (*A*) Results are expressed as the percent injected radioactivity. (*B*) Recovered radioactivity is normalized for tissue wet weight to give a measure of the concentration of radioactivity in the tissue. The miscellaneous category includes thymus, fat, and muscle. Results are the mean±SD for four rats (except S-LAP group, where n = 3). Tissues were taken at ~ 90 min after injection for samples with S-LAP, and ~ 30 min after injection for the others. The tissue distribution of S-LAP containing samples was similar at 30 and 90 min, but with a slightly higher concentration of label in the blood at the earlier time points.

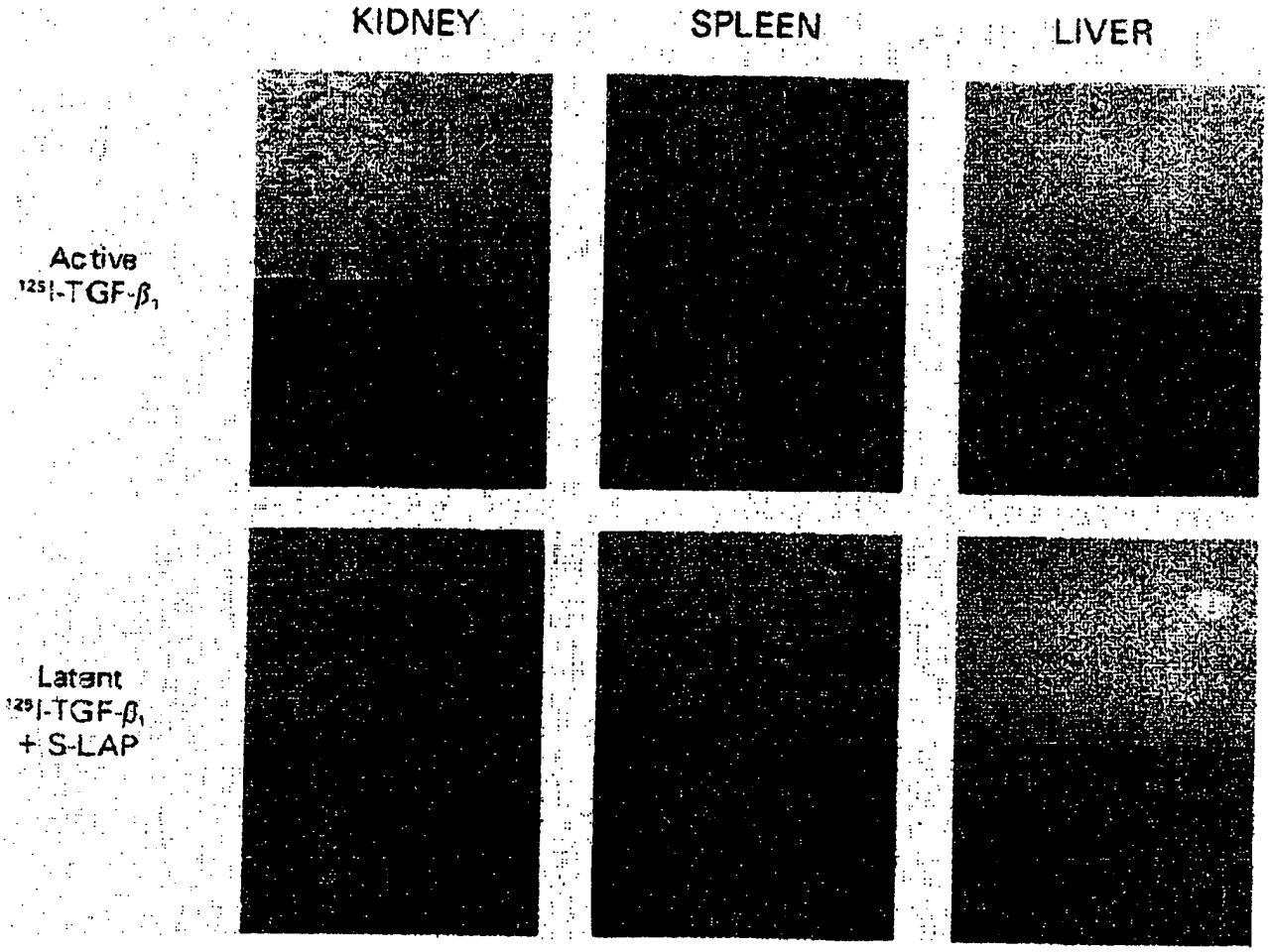


Figure 5. Autoradiography of tissue sections from rats injected with ^{125}I–TGF-β1 alone or complexed with S-LAP. 5-μm sections were rehydrated through xylene and graded alcohols, and then dipped in Kodak NTB2 emulsion. After 5 wk, slides were developed and stained with hematoxylin & eosin. Brightfield and darkfield photographs were taken with a Zeiss Axioplan microscope at ×100. For details, see text. Higher magnification photographs show that the refractility seen in the spleen of animals treated with latent TGF-β is due to blood cells and not to ^{125}I (not shown). In all other instances, the refractile material represents silver grains indicating the presence of radioiodinated material.

the extracellular fluid or some organ sink. In contrast, the V_d for latent TGF-β1 formed with S-LAP was ~ 17 ml, suggesting that the latent complex is largely confined to the circulatory system (Table I). This was confirmed by analysis of the recovery of radioactivity in the various organs upon killing the animal. For active TGF-β1, more radioactivity was recovered in the liver than in any other organ, and only ~ 4% was recovered in the plasma at 20 min after injection (Fig. 4 *A*). By contrast, for latent TGF-β1 formed with S-LAP, only 8% of the radioactivity was recovered in the liver as late as 90 min after injection, and all other organs had ≤ 2%, whereas 62.9±6.1% was recovered in the plasma. The recovery of active TGF-β1 in the liver is consistent with previously published data suggesting that active TGF-β1 is cleared by this organ (14). Interestingly, while the liver had the largest amount of TGF-β, normalization of recovered radioactivity to organ wet weight indicated that ^{125}I–TGF-β1 was actually most concentrated in the lungs. The concentration in kidneys, spleen, and liver were comparable to each other and approximately half that in the lungs (Fig. 4 *B*). By contrast, very little of the ^{125}I–TGF-β1 in the latent complex formed with S-LAP was found in any organ, as long as 90 min after injection, confirming that the majority of this complex does not pass out of the circulation (Fig. 4 *B*). As expected, the latent complex formed with U-LAP or with desialylated S-LAP was recovered almost exclusively in the liver.

Localization of iodinated material within organs. The ^{125}I in the various organs was further localized by autoradiography of organ slices, and the results are shown in Figs. 5 and 6. For animals injected with active TGF-β (^{125}I–TGF-β alone), the pattern of grains in the liver was fairly uniform, suggesting association of the TGF-β1 with the hepatocytes, consistent with the proposal of Coffey et al. that hepatocytes, but not nonparenchymal cells, can take up ^{125}I–TGF-β1 (14). In the kidney, labeled material was concentrated in the glomeruli, and in the lung it was found throughout the interstitium. The ^{125}I in the spleen localized to an area surrounding the white pulp, but was largely excluded from the red pulp and the center of the white pulp. By contrast, in animals that had been injected with the fully sialylated latent TGF-β1, the majority of

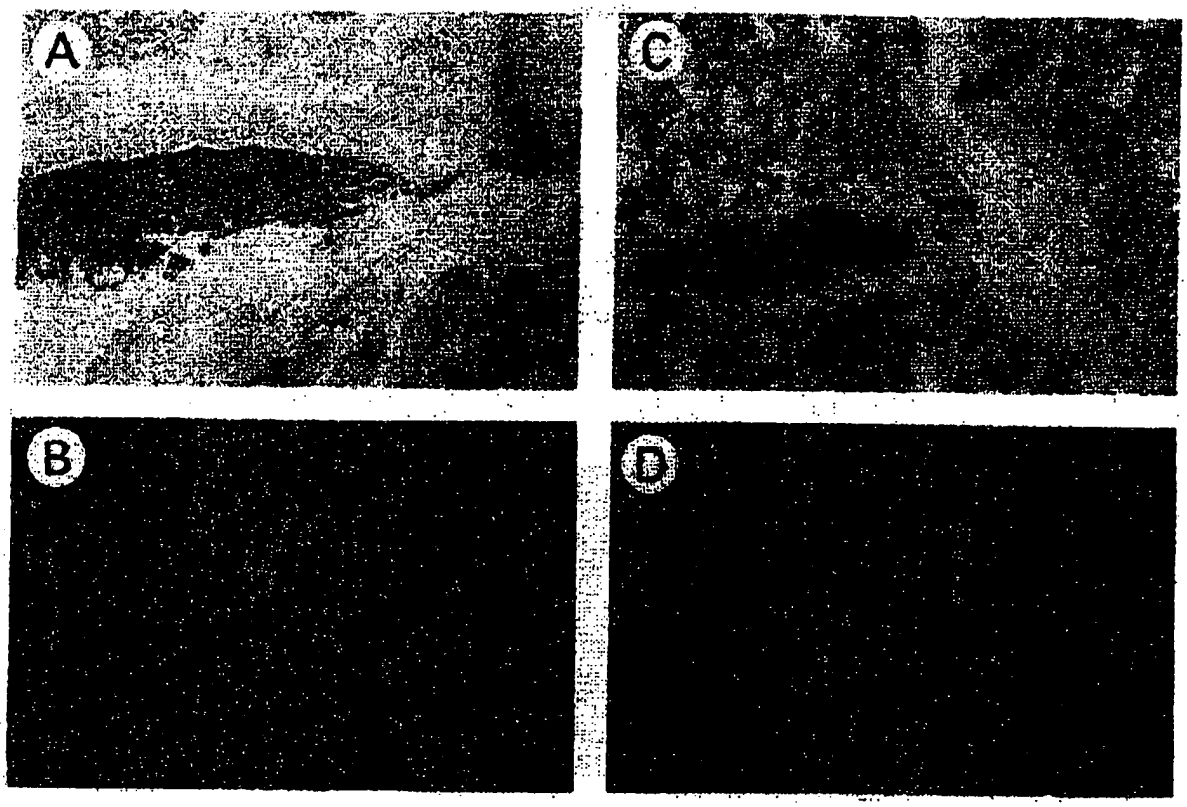


Figure 6. Autoradiography of lung sections from rats injected with ^{125}I–TGF-β alone or complexed with S-LAP. Sections were treated as for Fig. 5. *A* and *B* are from animals injected with latent TGF-β (^{125}I–TGF-β + S-LAP), and *C* and *D* are from animals injected with active TGF-β (^{125}I–TGF-β alone). *A* and *C* are brightfield, and *B* and *D* the corresponding darkfield exposures.

the iodinated material was associated with the blood, and there was little labeling above background in any organ. The only significant concentration of label was observed in the wall of a large blood vessel in the lung (Fig. 6 *B*). Since no sections from the other organs contained large blood vessels, it is not clear if this is a feature specific to the lung. However, it is apparent that in addition to distributing at low levels to most organs (see Fig. 4), the latent complex may concentrate in certain sites outside the circulatory system.

Degradation of circulating TGF-β1. By 15 min after injection of active ^{125}I–TGF-β1, only 53±17% of the radioactivity recovered from the plasma was precipitatable by TCA (Fig. 7). This indicates that active TGF-β1 is rapidly degraded and that some of the degradation products either remain in or reenter the circulation. By contrast, the ^{125}I–TGF-β1 in the latent complex formed with S-LAP appeared to be protected from this degradation process, with < 5% of the circulating material degraded, as long as 90 min after injection.

Analysis by SDS-PAGE of the iodinated material circulating in the plasma 30 min after injection of the animals with latent TGF-β (^{125}I–TGF-β + S-LAP), confirmed that there is little degradation of the ^{125}I–TGF-β in the complex, as indicated by the absence of bands below the band corresponding to intact TGF-β at 25 kD (Fig. 8, lane *3*). Chemical cross-linking of the labeled material in the plasma before electrophoresis gives a band at 100 kD (Fig. 8, lanes *2* and *4*), characteristic of the recombinant latent complex (Fig. 8, lane *5*). This suggests that the latent complex is circulating in the form in which it was injected. Note that chemical cross-linking reactions of this type are low efficiency, so only a fraction of the complex is crosslinked and stable to the denaturing conditions of electrophoresis. In the case of animals injected with active TGF-β (^{125}I–TGF-β alone), the very rapid clearance results in a low ratio of radioactivity to total plasma protein which precludes analysis of these samples by SDS-PAGE.

Extent of degradation of ^{125}I-TGF-β in tissues. To determine whether the radioactivity seen in the tissue autoradio-

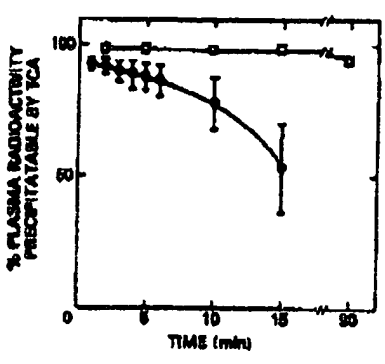


Figure 7. Degradation of circulating ^{125}I–TGF-β1. Degradation of circulating ^{125}I–TGF-β1 alone, or in latent complexes with LAP species, was monitored by the change in TCA precipitability of the radioactivity in the plasma with time. Data are mean±SD for three determinations in each group. Active TGF-β (●); latent TGF-β (TGF-β + S-LAP) (○).

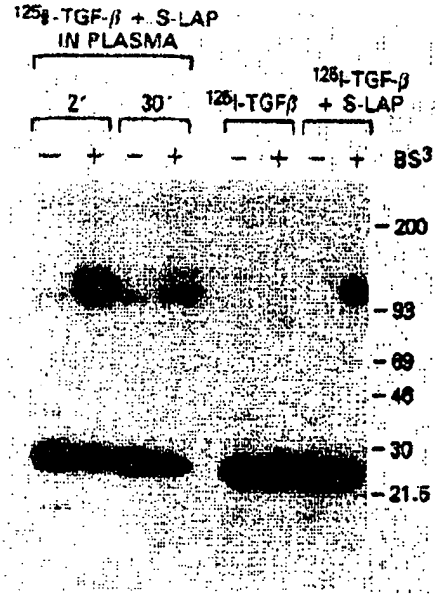


Figure 8. SDS-PAGE analysis of iodinated material recovered from rat plasma after injection with latent TGF-β. Plasma was prepared from rats 2 and 30 min after injection with latent TGF-β (^{125}I-TGF-β + S-LAP). After a 3× dilution with PBS, the plasma samples were treated with the crosslinking agent BS^3 or a buffer control as described in Methods, and samples were analyzed on 3–15% gradient gels under nonreducing conditions. Radioactive bands were visualized by autoradiography. Lanes 4–8 show the expected bands obtained on crosslinking an equivalent amount of purified active or latent TGF-β.

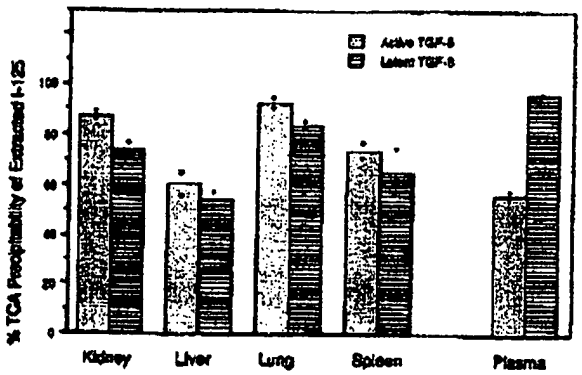


Figure 9. Effect of latent complex formation on the extent of degradation of TGF-β recovered from different organs. 30 min after injection with either latent TGF-β (^{125}I-TGF-β + S-LAP) or active TGF-β (^{125}I-TGF-β alone), animals were killed and the organs were immediately extracted with acid ethanol as described in Methods. The TCA precipitability of the radioactivity recovered from each organ was determined and used as a measure of the extent of degradation of TGF-β in that tissue. Bars represent the mean of two separate experiments, with the individual results indicated by the dots. The TCA precipitability of the iodinated material remaining in the plasma 30 min after injection is also given as a control. This shows that the extent of degradation of iodinated material in the various organs is not simply a passive reflection of that in the plasma.

graphs corresponded to intact or degraded ^{125}I-TGF-β, animals were killed after 30 min and organs were immediately extracted with acid-ethanol. The fraction of the extract that was precipitable by TCA was then determined. The results shown in Fig. 9 indicate that 70–90% of the ^{125}I recovered from lung and kidney was TCA precipitable, suggesting relatively low rates of degradation in these organs. More degradation was evident in the spleen, and particularly in the liver, where only 50–60% of the recovered ^{125}I was TCA precipitable. The TCA-soluble material probably represents iodotyrosine, small iodinated peptides, and possibly some free iodine released by the action of tissue dehalogenases. The high protein content of these extracts relative to the amount of radioactivity prevented SDS-PAGE analysis of the extracted radioactivity.

Although much less radioactivity distributed to the tissues of animals injected with latent TGF-β (^{125}I-TGF-β + S-LAP), the fraction that was TCA precipitable in each organ was very similar to that observed when active TGF-β (^{125}I-TGF-β alone) was injected. This suggests that the various tissues may process the two forms of TGF-β in a similar manner once it is delivered to them. Alternatively, it is possible that the latent TGF-β becomes activated in some way on leaving the circulation. The observation that active, not latent, TGF-β is recovered from the conditioned medium of endothelial cell and pericyte cocultures is interesting in this context (8). Since no antibodies are available for immunoprecipitation of the latent complex or S-LAP, it is not yet possible to determine whether latent TGF-β is taken up by organs as the complex or in the activated form. However, it is clear that regardless of whether the TGF-β is circulating in the active or the latent form, once it is taken up by the liver and spleen, it is degraded at a similar rate.

Discussion

The data show that recombinant latent TGF-β1 has a greatly extended plasma half-life, when compared with active TGF-β1, and a radically different tissue distribution. The rapid clearance of active TGF-β1 and its targeting predominantly to the liver, agree with a previous report (14). We have additionally shown a significant uptake of active TGF-β by lungs, spleen, and kidneys. The overall distribution pattern is similar to that observed for clearance of α-2-macroglobulin (α_2M) (22, 23) and is consistent with the proposal that active TGF-β1 may complex with α_2M and be cleared in this form (24). We have previously shown that latent TGF-β1, unlike active TGF-β1, is unable to bind to α_2M (10). Thus our demonstration of a much longer half-life for latent TGF-β1 suggests that an important role of the TGF-β1 LAP may be to protect TGF-β1 from being scavenged by circulating α_2M.

The extended plasma half-life of latent TGF-β1 has important physiological and clinical implications. From a physiological standpoint, the active TGF-β1 would be expected to act locally, close to its site of synthesis, since on leaving the cell it would be rapidly bound either by the ubiquitous TGF-β1 cell surface binding proteins, or by α_2M in the interstitial fluid. However, latent TGF-β1, which is unable to bind to the

TGF-β1 receptors or to α_2M, would be able to diffuse away from its site of synthesis and circulate to more distant target organs. Thus, whereas active TGF-β1 would have an autocrine/paracrine action, latent TGF-β1 might have a more endocrine mode of action. It is interesting to note that while most cells secrete TGF-β1 in the latent form, in a few instances, TGF-β1 is secreted in an active form (25, 26). In this way cells may be able to exert some control over the range of action of the TGF-β1 that they secrete. Normal human plasma contains significant levels of TGF-β from non-platelet sources (1.5±0.6 ng/ml (n = 10); Wakefield, L. M., unpublished data). The plasma TGF-β is latent, and while the lack of sensitive reagents for detecting latent TGF-β complexes so far precludes identification of the type of latent complex, it is clear that latent TGF-β does circulate in normal individuals and may play an important role in mediating regulatory interactions between organs.

In a clinical setting, the longer half-life of the latent TGF-β1 complex could be advantageous in greatly extending the duration of action of a single injected dose of the factor. Furthermore, the demonstration that systemically administered active TGF-β1 becomes concentrated in the lungs, kidneys, and spleen, as well as the liver, is potentially problematic in relation to the known ability of TGF-β1 to cause fibrosis in vivo (27). It will be important to determine whether the TGF-β1 delivered to these sites can exert any biological effect. In contrast, correctly sialylated latent TGF-β1 did not accumulate appreciably in any one organ, but seemed to distribute at low levels among them all, with the only significant concentration of latent TGF-β1 in any organ being to the wall of a large blood vessel in the lung.

It is not yet known which cell types are capable of activating latent TGF-β1 in vivo, but clearly this will determine the clinical targets for the latent complex, since it appears to distribute at low levels to most perfused organs. When large quantities of purified recombinant latent TGF-β1 become available, it should be possible to infuse animals with the material and look for effects on different organ systems. We have shown that the degree of sialylation of the latent complex is critical in determining both half-life and organ distribution. Since recombinant expression systems are variable in the degree to which they sialylate proteins (19), and all clinical work is likely to be done with recombinant material, it will be vital for investigators using this material to confirm that it has an adequate degree of sialylation. Furthermore, while native platelet and recombinant latent TGF-β1 are very similar in many in vitro properties (13, 20), it will be important to compare these in vivo. This may provide clues as to the role of the additional 135-kD protein in the natural latent TGF-β1, and will allow assessment of the degree to which the recombinant form will mimic or improve upon the natural complex in a clinical setting.

References

1. Roberts, A. B., K. C. Flanders, P. Kondaiah, N. L. Thompson, E. Van Obberghen-Schilling, L. M. Wakefield, P. Rossi, B. De Crombrugghe, U. Heine, and M. B. Sporn. 1988. Transforming growth factor-β: biochemistry and roles in embryogenesis, tissue repair and remodeling and carcinogenesis. *Recent Prog. Horm. Res.* 44:157–197.

2. Mustoe, T. A., G. F. Pierce, A. Thomason, P. Gramates, M. B. Sporn, and T. F. Deuel. 1987. Accelerated healing of incisional wounds in rats induced by transforming growth factor-β. *Science (Wash. DC).* 237:1333–1336.

3. Gooy, H., J. R. Keller, T. Back, D. L. Longo, F. W. Ruscetti, and R. H. Wiltrout. 1989. Inhibition of early murine hemopoietic progenitor cell proliferation after in vivo locoregional administration of transforming growth factor-β. *J. Immunol.* 143:877–880.

4. Twardzik, D. R., J. E. Ranchalis, J. M. McPherson, Y. Ogawa, L. Gentry, A. Purchio, E. Plata, and G. J. Todaro. 1989. Inhibition and promotion of differentiated-like phenotype of a human lung carcinoma in athymic mice by natural and recombinant forms of transforming growth factor-β. *J. Natl. Cancer Inst.* 81:1182–1185.

5. Lawrence, D. A., R. Pircher, C. Krycève-Martinerie, and P. Jullien. 1984. Normal embryo fibroblasts release transforming growth factors in a latent form. *J. Cell. Physiol.* 121:184–188.

6. Wakefield, L. M., D. M. Smith, T. Masui, C. C. Harris, and M. B. Sporn. 1987. Distribution and modulation of the cellular receptor for transforming growth factor-β. *J. Cell Biol.* 105:965–975.

7. Lyons, R. M., J. Keski-Oja, and H. L. Moses. 1988. Proteolytic activation of latent transforming growth factor-β from fibroblast-conditioned medium. *J. Cell Biol.* 106:1659–1665.

8. Antonelli-Orlidge, A., K. B. Saunders, S. R. Smith, and P. A. d'Amore. 1989. An activated form of transforming growth factor-β is produced by cocultures of endothelial cells and pericytes. *Proc. Natl. Acad. Sci. USA.* 86:4544–4548.

9. Sato, Y., and D. B. Rifkin. 1989. Inhibition of endothelial cell movement by pericytes and smooth muscle cells: activation of a latent transforming growth factor-β1-like molecule by plasmin during coculture. *J. Cell Biol.* 109:309–315.

10. Wakefield, L. M., D. M. Smith, K. C. Flanders, and M. B. Sporn. 1988. Latent transforming growth factor-β from human platelets: a high molecular weight complex containing precursor sequences. *J. Biol. Chem.* 263:7646–7654.

11. Miyazono, K., U. Hellman, C. Wernstedt, and C.-H. Heldin. 1988. Latent high molecular weight complex of transforming growth factor-β1: purification from human platelets and structural characterization. *J. Biol. Chem.* 263:6407–6415.

12. Gentry, L. E., N. R. Webb, J. Lim, A. M. Brunner, J. E. Ranchalis, D. R. Twardzik, M. N. Lioubin, H. Marquardt, and A. F. Purchio. 1987. Type 1 transforming growth factor-β: amplified expression and secretion of mature and precursor polypeptides in Chinese hamster ovary cells. *Mol. Cell. Biol.* 7:3418–3427.

13. Wakefield, L. M., D. M. Smith, S. Broz, M. Jackson, A. D. Levinson, and M. B. Sporn. 1989. Recombinant TGF-β1 is synthesized as a two-component latent complex that shares some structural features with the native platelet latent TGF-β1 complex. *Growth Factors.* 1:203–218.

14. Coffey, R. J. Jr., L. J. Kost, R. M. Lyons, H. L. Moses, and N. F. LaRusso. 1987. Hepatic processing of transforming growth factor-β in the rat: uptake, metabolism, and biliary excretion. *J. Clin. Invest.* 80:750–757.

15. Frolik, C. A., L. M. Wakefield, D. M. Smith, and M. B. Sporn. 1984. Characterization of a membrane receptor for transforming growth factor-β in normal rat kidney fibroblasts. *J. Biol. Chem.* 259:10995–11000.

16. McCaffrey, T. A., D. J. Falcone, C. F. Brayton, L. A. Agarwal, F. G. P., Welt, and B. B. Weksler. 1989. Transforming growth factor-β activity is potentiated by heparin via dissociation of the transforming growth factor-β/alpha$_2$macroglobulin inactive complex. *J. Cell Biol.* 109:441–448.

17. Urlaub, G., and L. Chasin. 1980. Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity. *Proc. Natl. Acad. Sci. USA.* 77:4216–4220.

18. Simonsen, C., and A. D. Levinson. 1983. Isolation and expression of an altered mouse dihydrofolate reductase cDNA. *Proc. Natl. Acad. Sci. USA.* 80:2495–2499.

19. Siminovitch, L. 1985. Mechanisms of genetic variation in Chinese hamster ovary cells. *In* Molecular Cell Genetics, XVII + 93P. M. M. Gottesman, editor. John Wiley and Sons, Inc., New York. 869–880.

20. Brown, P. D., L. M. Wakefield, A. D. Levinson, and M. B. Sporn. 1990. Physicochemical activation of recombinant latent transforming growth factor-βs 1, 2 and 3. *Growth Factors.* 3:35–43.

21. Ashwell, G., and J. Harford. 1982. Carbohydrate-specific receptors of the liver. *Annu. Rev. Biochem.* 51:531–554.

22. Okubo, H., H. Ishibashi, J. Shibata, K. Tsuda-Kawamura, and T. Yanase. 1984. Distribution of alpha$_2$Macroglobulin in normal, inflammatory, and tumor tissues in rats. *Inflammation.* 8:171–179.

23. Feldman, S. R., M. R. Rosenberg, K. A. Ney, G. Michalopoulos, and S. V. Pizzo. 1985. Binding of alpha$_2$Macroglobulin to hepatocytes: mechanism of in vivo clearance. *Biochem. Biophys. Res. Commun.* 128:795–802.

24. O'Connor-McCourt, M. D., and L. M. Wakefield. 1987. Latent transforming growth factor-β in serum: a specific complex with alpha$_2$macroglobulin. *J. Biol. Chem.* 262:14090–14099.

25. Assoian, R. K., B. E. Fleurdelys, H. C. Stevenson, P. J. Miller, D. K. Madtes, E. W. Raines, R. Ross, and M. B. Sporn. 1987. Expression and secretion of type β transforming growth factor by activated human macrophages. *Proc. Natl. Acad. Sci. USA.* 84:6020–6024.

26. Knabbe, C., M. E. Lippman, L. M. Wakefield, K. C. Flanders, A. Kasid, R. Derynck, and R. B. Dickson. 1987. Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell.* 48:417–428.

27. Roberts, A. B., M. B. Sporn, R. K. Assoian, J. M. Smith, N. S. Roche, L. M. Wakefield, U. I. Heine, L. A. Liotta, V. Falanga, J. H. Kehrl, and A. S. Fauci. 1986. Transforming growth factor type β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc. Natl. Acad. Sci. USA.* 83:4167–4171.

EXHIBIT 2

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 266, No. 33, Issue of November 25, pp. 22290-22296, 1991
Printed in U.S.A.

Interaction of Transforming Growth Factor-β1 with α2-Macroglobulin

ROLE IN TRANSFORMING GROWTH FACTOR-β1 CLEARANCE*

(Received for publication, July 18, 1991)

Anie Philip‡ and Maureen D. O'Connor-McCourt§

From the Section of Protein Engineering, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec H4P 2R2, Canada

It has been widely assumed that the interaction of transforming growth factor-β1 (TGF-β1) with its serum-binding protein, α2-macroglobulin (α2M), mediates the rapid clearance of TGF-β1 from the circulation. To test this, we have analyzed the effect of TGF-β1 binding on the conformational state of α2M. Our results demonstrate that the binding of TGF-β1 to α2M does not lead to the conformational change in the α2M molecule that is required for the clearance of the α2M·TGF-β1 complex via the α2M receptor. Furthermore, endogenous TGF-β1 is associated with the conformationally unaltered slow clearance form of α2M. Clearance studies in mice show that the half-life of ^{125}I-TGF-β1 in the circulation (1.6 ± 0.71 min) is not affected by blocking the α2M receptor with excess conformationally altered α2M. These results suggest that TGF-β1 is rapidly cleared from the circulation after injection by a pathway not involving α2M.

Transforming growth factor-β_1 (TGF-β1)[1] is a 25-kDa homodimeric peptide that modulates the growth and/or differentiation of almost every cell type (for review, see Ref.1). The fact that almost all cells have receptors for TGF-β suggests that it may have a broad spectrum of target tissues. TGF-β1 is a highly conserved peptide: its amino acid sequence is virtually identical in human, monkey, cow, pig, and chicken. This suggests a commonality in the nature of its action. The recent interest in research on TGF-β1 is due in part to its potential for therapeutic applications. It is implicated in inflammation and tissue repair (2, 3), formation of bone and cartilage (4, 5), modulation of the immune system (6), and carcinogenesis (7).

TGF-β1 is secreted from cells (8, 9) and released from degranulating platelets (10, 11) in a biologically inactive (latent) high molecular weight form. The TGF-β1 present in serum also exists in a latent form (12). Treatment *in vitro* of the cell-secreted, platelet-released, and serum latent forms of TGF-β1 with acid, alkali, or chaotropic agents can liberate the active form of TGF-β1 (13, 14). The mechanism involved in the activation *in vivo* is not known, but may involve proteases or other cell-specific interactions (14–16). The latent form of TGF-β1 released from platelets (10, 11) has recently been shown to consist of three components: (*i*) mature TGF-β1, (*ii*) a 40-kDa protein consisting of the remainder of the precursor, and (*iii*) a 125–160-kDa protein distinct from known proteins. The cell-secreted form has only the first two components (17, 18). We have previously identified the latent form in serum as an α_2-macroglobulin-TGF-β complex (12).

The nature of the biologically inactive forms of TGF-β1 and the physiological consequences of the formation of such complexes are central to the understanding of the control of TGF-β1 action. Since all cells so far studied have TGF-β1 receptors, it has been suggested that a major point of regulation of TGF-β1 action is at the level of TGF-β1 availability (19). Moreover, it has been proposed that the secreted form of latent TGF-β1 is a cellular delivery complex and that the circulating α2M-bound TGF-β1 of serum is a clearance complex (1, 20).

The general assumption that the binding of TGF-β1 to α2M represents a clearance mechanism for TGF-β1 from the circulation is based on the known fate of proteases bound to α2M. Protease binding to α2M is initiated by a specific limited proteolysis of the "bait region" (a particularly exposed peptide sequence) of α2M. This is followed by activation and cleavage of internal thiol ester bonds in α2M and covalent binding of the protease to α2M. This results in a large conformational change in the α2M molecule, with the concomitant exposure of previously concealed receptor recognition sites and subsequent rapid clearance of the α2M-protease complex from the circulation (21, 22). A similar conformational change can be induced in α2M by treatment with small nucleophiles such as methylamine ($MeNH_2$), which also results in the exposure of receptor-binding sites and the rapid clearance of the α2M·$MeNH_2$ complex (23, 24). The conformationally altered form of α2M is structurally more compact and can be readily distinguished from the native unaltered form on nondenaturing PAGE as an electrophoretically faster mobility band (25).

If TGF-β1 is cleared through the α2M pathway, TGF-β1 binding would be expected to induce the same conformational change in the α2M molecule as the proteases. To examine whether the binding of TGF-β1 to α2M represents a physiologically relevant clearance mechanism, we have analyzed the effect of TGF-β1 binding on the conformational state of α2M using serum and purified α2M. In addition, we have studied the *in vivo* clearance of TGF-β1 in the mouse. Our results demonstrate that the rapid clearance of TGF-β1 from the circulation after injection is not due to its interaction with α2M.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a Natural Sciences and Engineering Research Council visiting fellowship. Present address: Dept. of Animal Science, McGill University, MacDonald College, 21, 111 Lakeshore Rd. Quebec, H9X ICO, Canada. Tel.: 514-398-7973; Fax: 514-398-7964.

§ To whom correspondence should be addressed: Biotechnology Research Inst., National Research Council of Canada, 6100 Royalmount Ave., Montréal, Quebec H4P 2R2, Canada. Tel.: 514-496-6382; Fax: 514-496-6232.

[1] The abbreviations used are: TGF-β1, transforming growth factor-β1; α2M, α2-macroglobulin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; $MeNH_2$, methylamine; D-PBS, Dulbecco's phosphate-buffered saline; BS^3, bis(sulfosuccinimidyl) suberate; bFGF, basic fibroblast growth factor.

EXPERIMENTAL PROCEDURES

Materials—Na^{125}I was purchased from Amersham Corp. ^{14}C-Methylated protein molecular weight SDS-PAGE standards were from Bethesda Research Laboratories. Nonradioactive high and low molecular weight SDS-PAGE standards and glycine were from Bio-Rad. Methylamine hydrochloride, mercaptoethanol, bovine serum albumin (98–99% pure), Sigmacote, EDTA, chloronaphthol, H_2O_2 (30% solution), and soybean trypsin inhibitor were from Sigma. Chloramine T was from Fisher, and benzoyl-DL-arginine-*p*-nitroanilide HCl was from A & C American Chemicals. Bovine trypsin was from Boehringer Mannheim, and BS^3 was from Pierce Chemical Co. A PD-10 (disposable Sephadex G-25M) column and chelating Sepharose 6B were obtained from Pharmacia LKB Biotechnology Inc.; zinc chloride was from Anachemia; and nitrocellulose paper was from Schleicher & Schuell.

Citrated fresh human plasma used for the purification of α_2M was obtained from the Canadian Red Cross (Montreal). Serum and plasma used for binding studies were prepared using blood samples obtained from healthy nonpregnant women. The blood specimens were collected, refrigerated for a few hours, centrifuged at 4 °C, and stored frozen at −20 °C until analysis. Polyclonal turkey anti-TGF-β antiserum was generously provided by Dr. David Danielpour (National Cancer Institute, National Institutes of Health). The horseradish peroxidase-labeled rabbit anti-turkey antibody was purchased from Zymed Laboratories Inc. Highly purified TGF-β1 prepared from porcine platelets was obtained from R & D Systems. Nonpregnant female mice (8–12 weeks old) were obtained from Charles River Breeding Laboratories, Inc.

Iodination of TGF-β1—Porcine TGF-β1 (1 μg, carrier-free) was iodinated using the chloramine-T method of Frolik *et al.* (26) as modified by Ruff and Rizzino (27). Briefly, TGF-β1 dissolved in 15 μl of 30% (v/v) acetonitrile containing 0.1% trifluoroacetic acid was diluted with 10 μl of 1.5 M sodium phosphate (pH 7.4). 1 mCi (10 μl) of Na^{125}I (specific activity, 13.06 mCi/μg of iodine) was then added. The reaction was started by the addition of 5 μl of chloramine-T solution (20 μg/ml in 1.5 M sodium phosphate (pH 7.4)). The reaction mixture was mixed well and incubated at room temperature with the further addition of 5 μl of chloramine-T solution at 2 and 3.5 min. The reaction was stopped at 4.5 min by the addition of 20 μl of tyrosine (9 mg/ml in 50 mM sodium phosphate (pH 7.4), 200 μl of potassium iodide (10 mg/ml in 50 mM sodium phosphate (pH 7.4), and 200 μl of urea (1.2 g/ml in 1 M HCl). After removing aliquots for total counts and trichloroacetic acid precipitation, the reaction mixture was transferred to a PD-10 column pre-equilibrated with a solution of 4 mM HCl, 75 mM NaCl, and 0.1% (w/v) bovine serum albumin. Iodinated TGF-β1 was eluted with the same buffer into siliconized tubes containing 0.5 ml of 4 mM HCl containing 1% bovine serum albumin. The specific activity of labeled TGF-β1 was typically 2 μCi/pmol. The ^{125}I-TGF-β1 iodinated using the lactose peroxidase method (specific activity, 2.5 μCi/pmol) and some of the ^{125}I-TGF-β1 prepared using the chloramine-T method of Frolik *et al.* (26) were generously provided by Dr. Lalage Wakefield (National Cancer Institute, National Institutes of Health). The biological activity of iodinated TGF-β1 was tested by determining specific binding to 3T3 cells as described by Wakefield *et al.* (19).

Purification of α_2M—Human α_2M was purified as described by Imber and Pizzo (24) with minor modifications. The procedure was as follows. Two batches of human plasma of 250 ml each were dialyzed at 4 °C against 20 liters or more of deionized water for 72 h with frequent changes of water. The plasma was then centrifuged at 10,000 × *g* for 30 min to get rid of insoluble substances. The supernatant was dialyzed for 24 h at 4 °C against 20 liters of phosphate buffer (0.1 M sodium phosphate monobasic containing 0.8 M NaCl (pH 6.5)). The dialyzed plasma was brought to room temperature and applied to a pre-equilibrated zinc-Sepharose affinity column (20 × 2.6 cm). The affinity column was prepared and pre-equilibrated as follows. Chelating Sepharose 6B was washed with distilled water and packed in the column. The column was washed with a solution containing 0.05 M EDTA and 0.5 M NaCl (pH 7.0) and rewashed with distilled water. The column was equilibrated with aqueous $ZnCl_2$ (3 mg/ml, pH adjusted to 6.0), washed with a solution containing 0.25 M sodium acetate and 0.15 M NaCl (pH 5.0), and finally equilibrated in a buffer that consisted of 0.1 M sodium phosphate and 0.8 M NaCl (pH 6.5). After the addition of dialyzed plasma sample to the column, it was washed with equilibrating buffer until the A_{280} was <0.01. The column was then washed with a solution containing 0.02 M sodium phosphate and 0.15 M NaCl (pH 6.0). The bound protein was eluted from the column with elution buffer consisting of 0.01 M sodium acetate and 0.15 M NaCl (pH 5.0). The peak fractions were pooled. α_2M eluted between 150 and 350 ml under the conditions we used. The concentration of α_2M was determined spectrophotometrically using the value $A^{1\,cm,1\%}_{280\,nm} = 8.93$ (28). When purified α_2M was analyzed by SDS-PAGE under reducing conditions, a single band of 180 kDa was observed (Fig. 1*A*). Nondenaturing gel analysis of purified α_2M showed that within levels of detection, 100% of it was in the electrophoretically slow form as shown by Coomassie Brilliant Blue staining (Fig. 1*B*) and autoradiography of iodinated α_2M (Fig. 1*C*).

The biological activity of purified α_2M was tested by the soybean trypsin inhibitor-resistant bound trypsin activity assay as described by Ganrot (29). In this assay, when α_2M is reacted with trypsin, the unreacted trypsin (but not the α_2M-bound trypsin) is inhibited by soybean trypsin inhibitor. Benzoyl-DL-arginine-*p*-nitroanilide HCl, a small substrate of trypsin, when added can react with the α_2M-bound trypsin. The benzoyl-DL-arginine-*p*-nitroanilide HCl splitting activity as measured by absorbance at 410 nm was proportional to the amount of purified α_2M added (data not shown).

The purified α_2M pool was dialyzed against 40% glycerol in PBS (0.1 M sodium phosphate monobasic, 0.8 M NaCl (pH 6.5)). The dialyzed α_2M in glycerol was aliquoted and stored at −20 °C at a final concentration of 8.5 mg/ml. The glycerol was removed from α_2M on a PD-10 column prior to use.

Iodination of α_2M—Radioiodination of α_2M was performed by the lactoperoxidase method using the Enzymobead reagent as per the information provided by Bio-Rad. Iodinated α_2M was stored at 4 °C and used within 3 weeks. The specific activity was typically 3.2 μCi/pmol. ^{125}I-α_2M migrated on nondenaturing PAGE exclusively as the "slow" form (Fig. 1*C*).

Treatment of α_2M, Serum, and ^{125}I-α_2M with Trypsin—Serum, purified α_2M, or ^{125}I-α_2M was incubated at a trypsin:α_2M ratio of 1 μg:10 μg (*i.e.* three times the amount necessary to saturate α_2M) in 25 mM Tris (pH 8.0) at 37 °C for 2 h. The concentration of α_2M in undiluted serum was assumed to be 2.2 mg/ml (22). Unbound trypsin was inactivated by soybean trypsin inhibitor added at a concentration of 1 μg/μg of trypsin. The trypsin-treated samples were stored at 4 °C and used within 3 weeks.

Treatment of α_2M, Serum, and ^{125}I-α_2M with Methylamine—Serum, purified α_2M, or ^{125}I-α_2M was incubated with 200 mM methylamine HCl (final concentration) in 25 mM Tris (pH 8.0) at 37 °C

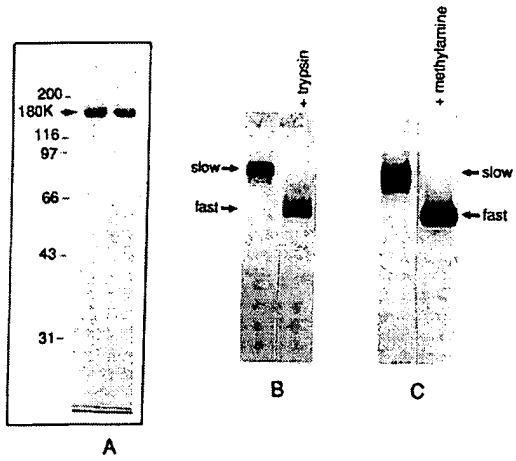


FIG. 1. **Migration pattern of purified α_2M as detected by Coomassie Brilliant Blue staining after SDS-PAGE (*A*), Coomassie Brilliant Blue staining after nondenaturing PAGE (*B*), and autoradiography after ^{125}I labeling of α_2M followed by nondenaturing PAGE (*C*).** *A*, SDS-PAGE of α_2M was done on a 3–11% linear gradient. The samples were heated at 37 °C in the presence of 100 mM mercaptoethanol. *B*, polyacrylamide gel electrophoresis of α_2M with and without trypsin pretreatment was done under nondenaturing conditions as described under "Experimental Procedures." The slow form corresponding to the conformationally unaltered state and the fast form corresponding to the trypsin-treated, conformationally altered state are indicated. *C*, ^{125}I-α_2M with and without methylamine pretreatment was subjected to electrophoresis under nondenaturing conditions followed by autoradiography. The slow and fast forms are indicated.

for 2 h. The methylamine-treated samples were stored at 4 °C and used within 3 weeks.

Preparation of α_2M·^{125}I-TGF-β1 Complex—The α_2M·^{125}I-TGF-β1 complex was prepared as described previously (12). Briefly, serum diluted to 8–16% (v/v) in D-PBS or purified α_2M, α_2M·MeNH$_2$, or α_2M-trypsin (100–200 μg/ml of D-PBS) was incubated overnight at 4 °C with ^{125}I-TGF-β1 at a final concentration of 0.4 nM. Where indicated, cross-linking was carried out as described previously (12). The samples were prepared for electrophoresis by the addition of an equal volume of 2 × concentrated SDS-PAGE sample buffer (0.125 M Tris-HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, and 0.01% bromphenol blue with or without 10% (v/v) mercaptoethanol) or nondenaturing PAGE sample buffer (0.082 M Tris, 0.08 M borate (pH 8.6), 20% (v/v) glycerol, and 0.01% (w/v) bromphenol blue). The samples were then subjected to SDS-PAGE or nondenaturing PAGE analysis.

Polyacrylamide Gel Electrophoresis—SDS-PAGE analysis was performed according to the method of Laemmli (30) using a 3–11% (w/v) linear gradient separating gel, 3% (w/v) stacking gel, and a Tris-Cl buffer system. The gels were calibrated using Bio-Rad low and high molecular weight standards. ^{14}C-Methylated protein standards were used for the calibration of radioactive bands. Nondenaturing PAGE was performed using the method described by Van Leuven *et al.* (31).

Western Blot—Plasma (diluted to 16% (v/v) in D-PBS) with and without trypsin pretreatment was separated by nondenaturing PAGE. The separated proteins were then immunoblotted essentially as described by Towbin *et al.* (32). Briefly, proteins were transferred to nitrocellulose (0.1 μM) overnight at 30 V followed by 1 h at 70 V. The nitrocellulose sheet was air-dried for a few minutes and blocked by incubation with 3% (w/v) skim milk powder in D-PBS for 30 min. It was then washed again in D-PBS and probed with turkey anti-TGF-β antiserum (1:50 dilution in D-PBS containing 0.05% (v/v) Tween 20) overnight at room temperature. After removal of the primary antibody, the nitrocellulose sheet was washed twice with D-PBS containing 0.05% (v/v) Tween 20, once with D-PBS containing 0.3% (w/v) skim milk powder, and once with D-PBS. It was then incubated overnight at room temperature with horseradish peroxidase-labeled rabbit anti-turkey antibody (1:500 dilution in D-PBS containing 0.05% (v/v) Tween 20). The sheet was washed as described above, and the samples were developed with a freshly prepared developing solution that consisted of a pinch of chloronaphthol dissolved in 20 ml of methanol, 80 ml of D-PBS, and 160 μl of 30% (v/v) H_2O_2.

Clearance Studies—Clearance studies were performed in 8–12-week-old CD-1 female mice from Charles River Breeding Laboratories, Inc. The samples were injected into the lateral tail veins of mice (unanesthetized) using a 1-ml tuberculin syringe and 28-gauge needle. Blood samples (25-μl aliquots) were collected from the retro-orbital venous plexus using calibrated hematocrit capillary tubes. The first blood sample was drawn within 10 s after injection. The remaining samples were drawn 1, 2, 3, 4, 5, 10, and 15 min after injection. Immediately after the last blood sample was taken, the mouse was killed by cervical dislocation, and various organs were collected. The radioactivity in blood samples and tissues was determined by a γ-counter. The radioactivity remaining in the circulation at different time periods was plotted as a percentage of the radioactivity in the first blood sample. The samples used for injection were as follows: (*i*) ^{125}I-TGF-β1, (*ii*) ^{125}I-TGF-β1 in the presence of an 800-fold molar excess of unlabeled α_2M·MeNH$_2$, (*iii*) ^{125}I-α_2M, (*iv*) ^{125}I-α_2M·MeNH$_2$, and (*v*) ^{125}I-α_2M·MeNH$_2$ in the presence of a 400-fold molar excess of unlabeled α_2M·MeNH$_2$. Whenever excess unlabeled α_2M·MeNH$_2$ was used, it was added just before (10–15 min) injection.

RESULTS

SDS-PAGE Analysis of ^{125}I-TGF-β1 Binding to Serum and Purified α_2M—In an earlier study, we showed that α_2M is the major serum protein that binds TGF-β1 and that this binding accounts for the latency of TGF-β1 in serum (12). In this study, the TGF-β1·α_2M interaction was further characterized by analyzing the complex on reducing and nonreducing gels both in the presence and absence of the covalent cross-linking agent BS^3.

When serum was cross-link-labeled by sequential incubation with ^{125}I-TGF-β1 and BS^3, ^{125}I-TGF-β1 co-migrated on nonreducing SDS-PAGE with the characteristic 300-kDa α_2M dimeric species and the 500-kDa α_2M tetrameric species (Fig. 2*A*, *lane 3*) and on reducing SDS-PAGE with the characteristic 180-kDa α_2M monomeric species (*lane 6*). When the same incubations were carried out in the absence of BS^3, the intensities of the 300-kDa band on nonreducing SDS-PAGE (*lanes 1* and *2*) and the 180-kDa band on reducing SDS-PAGE (*lanes 4* and *5*) were greatly diminished, but nonetheless detectable. Since the labeling was up to 100-fold higher in the presence of the cross-linking agent BS^3, the binding of TGF-β1 to α_2M appears to be predominantly noncovalent *in vitro*. The minor intrinsic covalent-like binding observed on SDS-PAGE in the absence of BS^3 was not due to an artifact of the chloramine-T procedure used for the iodination of TGF-β1 since these bands were also detectable when TGF-β1 iodinated by the lactoperoxidase method was used (*lanes 1 versus 2* and *4 versus 5*). Moreover, this binding was specific as suggested by >50% displacement of bound radioactivity by a 200-fold molar excess of unlabeled TGF-β1 (data not shown).

The covalent binding (*i.e.* that observed in the absence of BS^3) was greatly decreased by adding the reducing agent mercaptoethanol during SDS-PAGE (Fig. 2*A*, *lanes 1* and *2 versus 4* and *5*). This suggests that sulfhydryl bonds may be partially responsible for the covalent-like binding of TGF-β1 to α_2M *in vitro*. The reason for the decrease in radioactivity in the presence of reducing agent when the chemical cross-linker BS^3 was used is not clear (*lane 3 versus 6*). This was also observed (and discussed) in our previous study (12).

Virtually identical results were obtained when purified α_2M diluted to the same concentration as that expected in serum was affinity-labeled with ^{125}I-TGF-β1 in the presence of BS^3 (Fig. 2*B*). In the absence of BS^3, longer exposures of the film were required for detection of the 300-kDa band on nonreducing SDS-PAGE (*lanes 7 versus 9*) and the 180-kDa band on reducing SDS-PAGE (data not shown). This suggests that

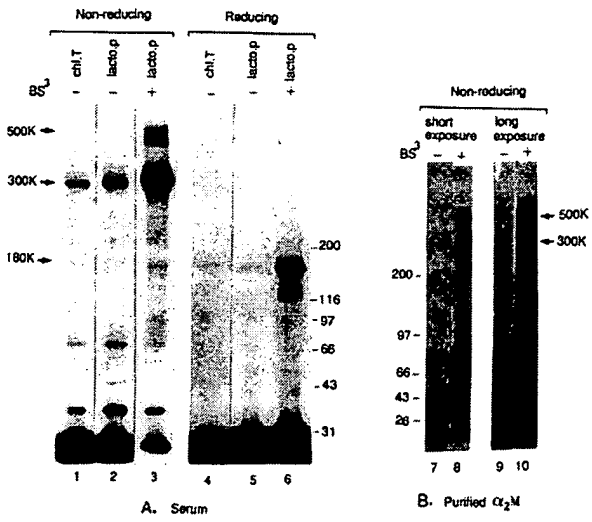


FIG. 2. **Affinity labeling of serum (*A*) and purified α_2M (*B*) with ^{125}I-TGF-β1.** *A*, human serum was diluted to 8% in D-PBS and labeled with ^{125}I-TGF-β1 (0.4 nM) followed by cross-linking with BS^3 where indicated (*lanes 3* and *6*). The samples were analyzed by 3–11% linear gradient SDS-PAGE in the absence (nonreducing, *lanes 1–3*) and presence (reducing, *lanes 4–6*) of 100 mM mercaptoethanol. The ^{125}I-TGF-β1 used in *lanes 2*, *3*, *5*, and *6* was iodinated using the lactoperoxidase procedure (*lacto.p*, lactoperoxidase; *chl.* T, chloramine T). *B*, purified α_2M (350 nM) was labeled with 0.4 nM ^{125}I-TGF-β1 followed by cross-linking with BS^3 where indicated (*lanes 8* and *10*). The samples were analyzed by 3–11% linear gradient SDS-PAGE in the absence of reducing agents. *Lanes 9* and *10* represent longer exposures of *lanes 7* and *8*, respectively.

the proportion of bound TGF-β1 that becomes intrinsically linked to α_2M in a covalent-like manner is less for purified α_2M as compared to serum. It may be that a serum factor is involved in the covalent-like binding of TGF-β1 to α_2M. However, the possibility that the purification procedure has altered the binding properties of α_2M cannot be ruled out.

Nondenaturing PAGE Analysis of ^{125}I-TGF-β1 Binding to Serum and Purified α_2M—The conformationally altered (rapid clearance) form of α_2M can be readily distinguished from the unaltered form as a faster mobility band on nondenaturing PAGE (25, 31). No conformationally altered α_2M could be detected in the purified α_2M preparation used in this study (Fig. 1, *B* and *C*). The results presented in Fig. 3 (*lanes 1* and *5*) show that ^{125}I-TGF-β1 binds to the conformationally unaltered (slow clearance) form of α_2M. When α_2M was conformationally altered by trypsin pretreatment, the binding of TGF-β1 was decreased (*lanes 1 versus 2* and *5 versus 6*). However, the methylamine-induced conformationally altered form of α_2M bound more TGF-β1 than the unaltered form (*lanes 3 versus 4* and *5 versus 7*). These observations are consistent with the data reported previously by Huang *et al.* (33) and very recently by LaMarre *et al.* (34).

Effect of TGF-β1 Binding on Conformational State of α_2M—To confirm that TGF-β1 does not induce the same conformational change in the α_2M molecule as protease, we tested the effect of equimolar concentrations of nonradioactive TGF-β1 on the conformational state of ^{125}I-labeled α_2M. By radiolabeling α_2M instead of TGF-β1, we should be able to sensitively detect conformational alterations that occur in the α_2M molecule upon TGF-β1 binding. Nondenaturing PAGE analysis of ^{125}I-α_2M incubated with an equimolar concentration of unlabeled TGF-β1 clearly showed that TGF-β1 binding did not induce the same conformational change in the α_2M molecule as proteases (Fig. 4). In addition, TGF-β1 at concentrations 10 times higher than α_2M did not cause the transition of the slow form to the fast form (data not shown). However, when the preformed ^{125}I-α_2M·TGF-β1 or α_2M·^{125}I-TGF-β1 complex was treated with trypsin, transition of the α_2M·TGF-β1 complex to the conformationally altered "fast" form was observed (Fig. 5). This shows that TGF-β1 binding to α_2M did not prevent subsequent protease binding and that trypsin did not release TGF-β1 from the α_2M·TGF-β1 complex.

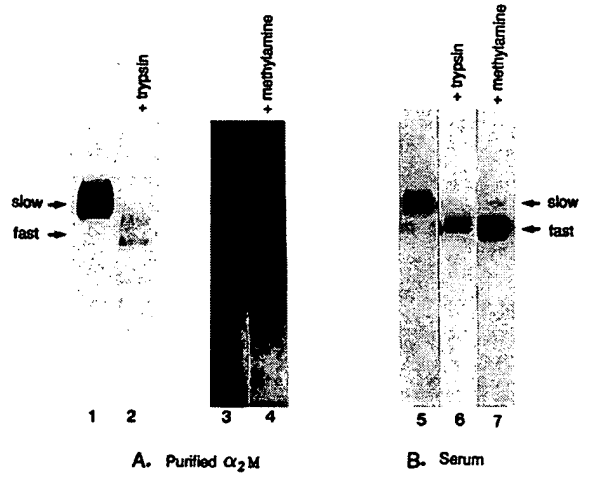


FIG. 3. **Effects of trypsin and methylamine treatment of purified α_2M (*A*) and serum (*B*) on complex formation with ^{125}I-TGF-β1.** *A*, purified α_2M (250 nM) left untreated (*lanes 1* and *3*) or treated with trypsin (*lane 2*) or methylamine (*lane 4*) was incubated with 0.4 nM ^{125}I-TGF-β1 and subjected to nondenaturing PAGE; *B*, serum diluted to 8% (v/v) with D-PBS left untreated (*lane 5*) or treated with trypsin (*lane 6*) or methylamine (*lane 7*) was incubated with 0.4 nM ^{125}I-TGF-β1 and subjected to nondenaturing PAGE conditions. Treatment of α_2M and serum with trypsin and methylamine was as described under "Experimental Procedures." The locations of the slow and fast forms of α_2M are indicated.

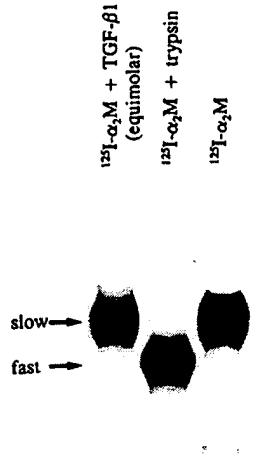


FIG. 4. **Effect of TGF-β1 binding on conformational state of α_2M.** ^{125}I-α_2M (0.4 nM) was incubated with an equimolar concentration of nonradioactive TGF-β1 and analyzed by nondenaturing PAGE and autoradiography. The migration pattern of ^{125}I-α_2M alone (slow form) and that of ^{125}I-α_2M treated with trypsin (fast form) are shown for comparison.

Endogenous TGF-β1 in Serum as Detected by Western Blot Analysis Following Nondenaturing PAGE—In view of the results presented up to this point, it became of interest to test whether endogenous TGF-β1 was also bound to the conformationally unaltered form of α_2M. Plasma was analyzed by nondenaturing PAGE, and endogenous TGF-β1 was located on Western blots using a specific anti-TGF-β1 antiserum. Endogenous TGF-β1 in plasma was associated with the conformationally unaltered slow form of α_2M (Fig. 6). Treatment of plasma with trypsin resulted in the transition of this endogenous α_2M·TGF-β1 complex to the fast form (Fig. 6). These results are consistent with the data obtained using exogenously added TGF-β1 and support the conclusion that TGF-β1 binding does not result in the conformational change that is necessary for the exposure of receptor recognition sites in the α_2M molecule. The amount of TGF-β1 detected in plasma after trypsin treatment appears to be higher than that in the untreated plasma. This most likely is due to the increased ability of the antibody to detect TGF-β1 when it is bound to conformationally altered α_2M.

Clearance Studies—The results obtained when radiolabeled derivatives of TGF-β1, α_2M, and α_2M·$MeNH_2$ were injected intravenously into mice in the presence and absence of excess unlabeled α_2M·$MeNH_2$ are shown in Fig. 7. ^{125}I-TGF-β1 was cleared with a half-life of 1.6 ± 0.71 min. ^{125}I-α_2M was removed from the circulation very slowly with a half-life of several hours. ^{125}I-α_2M·$MeNH_2$ (conformationally altered α_2M with the receptor recognition site exposed) was cleared with a half-life 5.3 ± 1.4 min. The clearance of ^{125}I-α_2M·$MeNH_2$ was

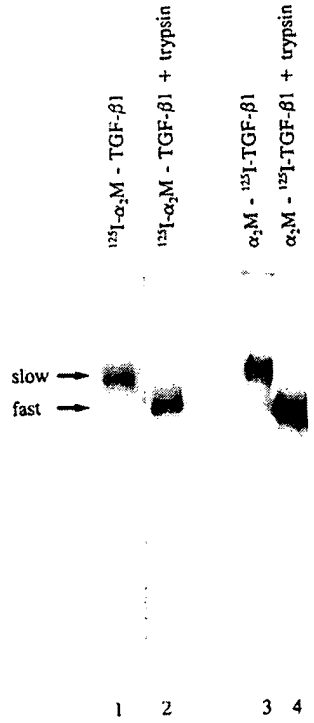


FIG. 5. **Effect of trypsin treatment on preformed α_2M•TGF-β1 complex.** In *lanes 1* and 2, ^{125}I-α_2M (0.4 nM) was incubated with nonradioactive TGF-β1 (0.4 nM). The preformed ^{125}I-α_2M·TGF-β1 complex was then left untreated (*lane 1*) or was treated with trypsin as described under "Experimental Procedures" (*lane 2*). In *lanes 3* and *4*, 0.4 nM ^{125}I-TGF-β1 was incubated with 250 nM nonradioactive α_2M. The preformed α_2M·^{125}I-TGF-β1 complex was then left untreated (*lane 3*) or was treated with trypsin as described above. The slow and fast forms of α_2M are indicated.

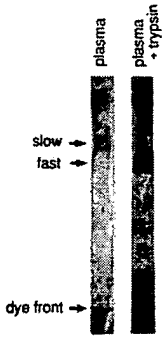


FIG. 6. **Immunoblot detection of TGF-β1 in plasma using anti-TGF-β1 antiserum following nondenaturing PAGE.** Plasma diluted to 16% (v/v) in D-PBS was left untreated or was treated with trypsin as described for serum under "Experimental Procedures." The proteins were separated by nondenaturing PAGE. Immunoblot analysis was performed using turkey anti-human TGF-β1 antiserum as described under "Experimental Procedures." The locations of the slow and fast forms of α_2M are indicated.

virtually blocked by the coinjection of a 400-fold molar excess of unlabeled α_2M·MeNH$_2$ as expected for a receptor-mediated clearance process. However, the coinjection of a 800-fold molar excess of unlabeled α_2M·MeNH$_2$ did not alter the half-life of ^{125}I-TGF-β1. The failure to influence TGF-β1 clearance appreciably by blockage of α_2M receptors with excess unlabeled α_2M·MeNH$_2$ (whereas such blockage clearly inhibited ^{125}I-α_2M·MeNH$_2$ clearance) together with the fact that the half-life of ^{125}I-TGF-β1 was significantly shorter than that of

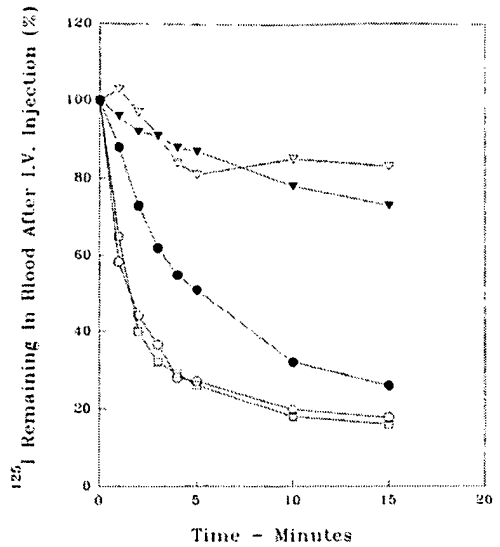


FIG. 7. **Clearance of radiolabeled derivatives of TGF-β1, α_2M, and α_2M•MeNH$_2$ in mouse.** Approximately 10^6 cpm of ^{125}I-α_2M (▽), ^{125}I-TGF-β1 (○), ^{125}I-α_2M·MeNH$_2$ (●), ^{125}I-α_2M·MeNH$_2$ in the presence of excess unlabeled α_2M·MeNH$_2$ (▼), or ^{125}I-TGF-β1 in the presence of excess unlabeled α_2M·MeNH$_2$ (□) was injected into the lateral tail veins of mice. Blood samples were collected, and radioactivity remaining in the circulation was calculated as described under "Experimental Procedures."

TABLE I

Distribution of injected ^{125}I-TGF-β1 and ^{125}I-α_2M·MeNH$_2$ in various organs

Approximately 10^6 cpm of ^{125}I-TGF-β1 or ^{125}I-α_2M·MeNH$_2$ was injected into the lateral tail veins of mice. After the final blood collection at 15 min, the mice were killed, and whole organs were counted. The radioactivity recovered in each organ was calculated as a percentage of total recovered radioactivity ± S.D.

Organ	Radioactivity recovered in each organ	
	^{125}I-TGF-β1	^{125}I-α_2M·MeNH$_2$
	%	
Liver	56 ± 9	91 ± 5
Lung	24 ± 6	3 ± 2
Kidney	15 ± 4	4 ± 3
Spleen	3 ± 1	1 ± 0
Heart	2 ± 1	1 ± 0

^{125}I-α_2M·MeNH$_2$ strongly suggest that the major pathway for TGF-β1 clearance from the circulation after injection is not through the α_2M receptor.

We also examined the organ distribution of cleared ^{125}I-TGF-β1 and ^{125}I-α_2M·MeNH$_2$ by removing organs immediately after the last blood collection and determining the radioactivity thus recovered. The distribution of ^{125}I-TGF-β1 was markedly different from that of ^{125}I-α_2M·MeNH$_2$ (Table I). In the case of ^{125}I-α_2M·MeNH$_2$, >90% of the recovered radioactivity was located in the liver. This is expected since the hepatocyte is the major cell type responsible for clearing α_2M·MeNH$_2$ through the α_2M receptor. In contrast, only 50–60% of the cleared ^{125}I-TGF-β1 was found in the liver, with significant amounts being located in the lung and kidney. In fact, taking into account the mass of the organs, the highest concentration of cleared ^{125}I-TGF-β1 was in the lung. These organ distribution results support the concept of an α_2M receptor-independent clearance route for TGF-β1.

DISCUSSION

The major finding in this study is that the binding of TGF-β1 to α_2M *in vitro* did not lead to the conformational change in the α_2M molecule that is necessary for clearance of the

α_2M·TGF-β1 complex through the α_2M receptor. Furthermore, our clearance studies in mice indicate that TGF-β1 is rapidly cleared from the circulation after injection by a pathway not involving the α_2M receptor. However, protease treatment *in vitro* of the endogenous or preformed exogenous α_2M·TGF-β1 complex resulted in the transition of the complex to the conformationally altered form, suggesting that α_2M has the potential to be involved in the clearance of TGF-β1 when proteases are also present.

Our results also indicate that when ^{125}I-TGF-β1 was added to serum, it became bound to α_2M in a predominantly noncovalent manner with minor reductant-sensitive and -resistant covalent-like binding components (Fig. 2). In contrast, we have previously shown that endogenous TGF-β1 is bound to α_2M in a predominantly covalent manner (12). It is conceivable that the three ways in which TGF-β1 binds to α_2M are steps in a sequential series as has been proposed for the binding of basic fibroblast growth factor to α_2M (35), *i.e.* noncovalent binding is followed by a reductant-sensitive linkage, which in turn leads to reductant-resistant covalent binding. Whereas these three steps may proceed smoothly *in vivo* with the result that TGF-β1 becomes predominantly covalently bound to α_2M, in the *in vitro* system, one or more of the components necessary for the covalent binding may be deficient or missing.

Previous reports (33, 36) have suggested that TGF-β1 binds solely to the conformationally altered form of α_2M. The discrepancy between our results and their observations may be explained if the α_2M preparations they used contained some conformationally altered α_2M that preferentially binds TGF-β1. The slow to fast conversion is known to occur readily if purification and storage conditions are not ideal. We have shown that our α_2M preparations contain no detectable conformationally altered α_2M (Fig. 1, *B* and *C*). While this manuscript was in preparation, LaMarre *et al.* (34, 37) reported that ^{125}I-TGF-β1 binds to the native form of α_2M, with methylamine pretreatment of α_2M increasing binding and trypsin pretreatment decreasing binding. These observations are in agreement with our results (Fig. 3). However, in their experiments, after incubation with native α_2M, most of the bound ^{125}I-TGF-β migrated more rapidly than the Coomassie Blue-stained native α_2M band. Accordingly, they qualify their interpretation of the binding of TGF-β1 to native α_2M by suggesting that the TGF-β1 may be selectively binding to trace amounts of conformationally altered α_2M species in their native α_2M preparation. We did not detect this shift in migration in our experiments. Also, we have addressed the question of the effect of TGF-β1 binding on the conformational state of α_2M directly by radiolabeling α_2M instead of TGF-β1 so that any conformational alteration induced by TGF-β1 in α_2M could be readily detected. Under these conditions, we detect no conformational change in α_2M after TGF-β1 binding (Fig. 4). In support of these *in vitro* experiments, we also showed by Western blot analysis that endogenous TGF-β1 in plasma is associated with the conformationally unaltered form of α_2M (Fig. 6). This demonstrates that, although TGF-β1 binds preferentially to certain conformationally transformed α_2M species *in vitro, i.e.* the methylamine- and plasmin-induced species (Fig. 3 and Ref. 34, respectively), the native α_2M·TGF-β1 complex is the most abundant form in the circulation. Since the fate of an α_2M-bound ligand is determined by the conformational state of the α_2M molecule, these data suggest that the α_2M·TGF-β1 complex is not cleared from the circulation through the α_2M pathway.

Further experiments, on the other hand, showed that treatment of the preformed α_2M·TGF-β1 complex with trypsin resulted in the transition of this complex to the conformationally altered form without any loss of bound TGF-β1 (Fig. 5). This conformational transition was also demonstrable by trypsin treatment of the endogenous α_2M·TGF-β1 complex as detected by Western blot analysis (Fig. 6). These results demonstrate that TGF-β1 binding does not prevent the subsequent interaction of protease with the α_2M·TGF-β1 complex and thus provides a mechanism for the clearance of TGF-β1 through the α_2M receptor under circumstances where active proteases are also present. Accordingly, under *in vivo* conditions such as inflammation or tissue damage, where active proteases are present and TGF-β1 is being released locally, the α_2M·TGF-β1 complex formed may be acted on by proteases to yield an α_2M·TGF-β1·protease complex that could be cleared through α_2M receptors. This mechanism for clearance of TGF-β1 through the α_2M receptor is distinct from that recently proposed by Lamarre *et al.* (34), whereby they suggest that TGF-β1 binding to the preformed α_2M-plasmin complex could result in TGF-β1 clearance through α_2M receptors. Both mechanisms could operate simultaneously *in vivo* depending on the presence of specific proteases.

The results from our clearance studies in mice support the conclusion that TGF-β1 is not cleared through α_2M receptors. The half-life that we observed for TGF-β1 in the circulation of mice (1.6 ± 0.71 min) is consistent with the half-life reported by Coffey *et al.* (38) in the rat (2.2 min) and recently by Lamarre *et al.* (37) in mice (~2 min). The clearance half-life of human α_2M·$MeNH_2$ in mice observed in this study (5.3 ± 1.4 min) is consistent with reported values (24, 37). The observation that the half-life of TGF-β1 was significantly shorter ($p < 0.001$) than that of α_2M·$MeNH_2$ together with the fact that the clearance of TGF-β1 was not blocked by the coinjection of excess unlabeled α_2M·$MeNH_2$ strongly support the proposal that TGF-β1 is cleared from the circulation after injection by a pathway independent of the α_2M receptor. A determination of the organ distributions of cleared ^{125}I-TGF-β1 and ^{125}I-α_2M·$MeNH_2$ gave further evidence that these two proteins are not being cleared from the circulation at identical sites. ^{125}I-α_2M·$MeNH_2$ became concentrated almost exclusively in the liver, as expected for an α_2M receptor-mediated process, whereas ^{125}I-TGF-β1 localized to the lung and kidney as well as the liver. Furthermore, we have recently determined the sites of uptake of ^{125}I-TGF-β1 from the circulation at the cellular level using light and electron microscopic autoradiography and have found that the hepatocyte is not the cell type responsible for TGF-β1 clearance from the circulation.[2]

The nature of the receptors through which injected TGF-β1 is rapidly cleared is not known. One of the already described TGF-β1 receptors (Type I or II or betaglycan) (1, 39) may be responsible; or alternatively, there may exist separate clearance receptors for TGF-β1. Also, it remains to be determined whether injected TGF-β1 binds to native α_2M in the circulation and is rapidly released at the site of clearance or whether clearance occurs before complex formation. The hypothesis that the α_2M receptor does not contribute to the clearance of free ^{125}I-TGF-β1 was recently alluded to by LaMarre *et al.* (37). However, the data presented in their paper concentrated on the clearance of TGF-β1 that was bound to a receptor-recognizable form of α_2M.

Other growth factors that have been reported to bind α_2M include platelet-derived growth factor (40, 41), nerve growth

[2] Philip, A., Dickson, K., Warshawsky, H., Bergeron, J. J. M., and O'Connor-McCourt, M. D. (1991) *Abstracts of the 73rd Annual Meeting of the Endocrine Society, Washington, D. C., June 19–23*, p. 150, abstr., Endocrine Society, Bethesda.

factor (42, 43), basic fibroblast growth factor (bFGF) (35), interleukin-1β (44), and interleukin-6 (45). The mode of binding (covalent *versus* noncovalent) of these growth factors to α_2M, the effect of the α_2M conformational state on growth factor binding, and the effect of α_2M on growth factor activity all vary depending on the growth factor involved. This is illustrated by comparing bFGF and TGF-β1. One might expect that these two growth factors would interact with α_2M in a similar manner since it has been reported that bFGF competes with TGF-β1 for binding to α_2M (35). However, there appears to be more differences than similarities in the way these two growth factors interact with α_2M. The similarities are: (*i*) both TGF-β1 and bFGF bind to α_2M *in vitro* in three modes, *i.e.* noncovalently, covalently in a reductant-sensitive manner, and covalently in a reductant-resistent manner (Fig. 2 and Ref 35); and (*ii*) reaction of α_2M with methylamine increased the binding of both TGF-β1 (Fig. 3) and bFGF (35). The differences include: (*i*) the binding is predominantly covalent for bFGF *in vitro* (35), whereas it is mainly noncovalent for TGF-β1 *in vitro* (Fig. 2); (*ii*) trypsin has no effect on bFGF binding (35), whereas it decreases TGF-β1 binding (Fig. 3); (*iii*) heparin, which binds bFGF, has no effect on the bFGF·α_2M interaction (35), but dissociates the α_2M·TGF-β1 complex (34, 46); (*iv*) the α_2M associated with the α_2M·bFGF complex exists only in the conformationally altered form (35), whereas the α_2M·TGF-β1 complex can be in either the native or conformationally altered form (Figs. 3 and 6); and (*v*) bFGF activity is decreased in the presence of α_2M, whereas α_2M has little or no effect on TGF-β1 bioactivity (12, 36).

We have proposed that, depending on the presence or absence of proteases, α_2M may or may not act as a clearance protein for TGF-β1. Under the circumstances where α_2M is not acting as a clearance protein, *i.e.* in the absence of proteases, it may actually serve as a protective carrier protein for TGF-β1. The Western blot analysis described in this study supports this hypothesis by demonstrating that there exists a pool of circulating TGF-β1 that is bound to the native form of α_2M. This observation is paradoxical, however, since our clearance studies showed that injected TGF-β1 was not stabilized (*i.e.* protected from clearance) by the endogenous native α_2M in the circulation. This inconsistency may be explained in part by the fact that endogenous α_2M-bound TGF-β is covalently linked, whereas injected TGF-β1 may form a reversible noncovalent complex with the endogenous α_2M. For the endogenous circulating pool of TGF-β1 to act in an endocrine manner, it is necessary to postulate that there exists a localized activation mechanism that would target TGF-β1 by releasing it from the endogenous α_2M·TGF-β1 complex at specific sites of action.

Acknowledgments—We thank Ailsa Leeloy for technical assistance with the intravenous injections in mice: Dr. David Danielpour for the gift of anti-TGF-β1 antiserum; Drs. David Thomas, Maria Debanne, and Jane Mitchell for critical review of this manuscript; and Lucie Morin for typing this manuscript.

REFERENCES

1. Roberts, A. B., and Sporn, M. B. (1989) *Handb. Exp. Pharmacol.* **95**, 419–472
2. Wahl, S. M., Hunt, D. A., Wakefield, L. M., McCartney-Francis, N., Wahl, L. M., Roberts, A. B., and Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 5788–5792
3. Mustoe, T. A., Pierce, G. F., Thomason, A., Gramates, P., Sporn, M. B., and Deuel, T. F. (1987) *Science* **237**, 1333–1336
4. Pfeilschifter, J., and Mundy, J. R. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 2024–2028
5. Centrella, M., McCarthy, T. L., and Canalis, E. (1988) *FASEB J.* **2**, 3066–3073
6. Goey, H., Keller, J. R., Back, T., Longo, D. L., Ruscetti, F. W., and Wiltrout, R. H. (1989) *J. Immunol.* **143**, 877–880
7. Twardzik, D. R., Ranchalis, J. E., McPherson, J. M., Ogawa, Y., Gentry, L., Purchio, A., Plata, E., and Todaro, G. J. (1989) *J. Natl. Cancer Inst.* **81**, 1182–1185
8. Lawrence, D. A., Pircher, R., Kryceve-Martinerie, C., and Jullien, P. (1984) *J. Cell. Physiol.* **121**, 184–188
9. Kryceve-Martinerie, C., Lawrence, D. A., Crochet, J., Jullien, P., and Vigier, P. (1985) *Int. J. Cancer* **35**, 553–558
10. Wakefield, L. M., Smith, D. M., Flanders, K. C., and Sporn, M. B. (1988) *J. Biol. Chem.* **263**, 7646–7654
11. Miyazono, K., Hellman, U., Wernstedt, C., and Heldin, C-H. (1988) *J. Biol. Chem.* **263**, 6407–6415
12. O'Connor-McCourt, M. D., and Wakefield, L. M. (1987) *J. Biol. Chem.* **262**, 14090–14099
13. Lawrence, D. A., Pircher, R., and Jullien, P. (1985) *Biochem. Biophys. Res. Cummun.* **133**, 1026–1034
14. Lyons, R. M., Keski-Oja, J., and Moses, H. L. (1988) *J. Cell Biol.* **106**, 1659–1665
15. Antonelli-Orlidge, A., Saunders, K. B., Smith, S. R., and d'Amore, P. A. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 4544–4548
16. Sato, Y., and Rifkin, D. B. (1989) *J. Cell Biol.* **109**, 309–315
17. Gentry, L. E., Webb, N. R., Lim, G. J., Brunner, A. M., Ranchalis, J. E., Twardzik, D. R., Lioubin, M. N., Marquardt, H., and Purchio, A. F. (1987) *Mol. Cell. Biol.* **7**, 3418–3427
18. Wakefield, L. M., Smith, D. M., Broz, S., Jackson, M., Levinson, A. D., and Sporn, M. B. (1989) *Growth Factors* **1**, 203–218
19. Wakefield, L. M., Smith, D. M., Masui, T., Harris, C. C., and Sporn, M. B. (1987) *J. Cell Biol.* **105**, 965–975
20. Wakefield, L. M., Thompson, N. L., Flanders, K. C., O'Connor-McCourt, M. D., and Sporn, M. B. (1988) *Ann. N. Y. Acad. Sci.* **551**, 290–298
21. Barrett, A. J., and Starkey, P. M. (1973) *Biochem. J.* **133**, 709–724
22. Sottrup-Jensen, L. (1987) in *Plasma Proteins* (Putkam, F. W., ed) 2nd Ed., Vol. 5, pp. 191–291, Acamemic Press, Orlando, FL
23. Sottrup-Jensen, L., Petersen, T. E., and Magnusson, S. (1980) *FEBS Lett.* **121**, 275–279
24. Imber, M. J., and Pizzo, S. V. (1981) *J. Biol. Chem.* **256**, 8134–8139
25. Barrett, A. J., Brown, M. A., and Sayers, C. A. (1979) *Biochem. J.* **181**, 401–418
26. Frolik, C. A., Wakefield, L. M., Smith, D. M., and Sporn, M. B. (1984) *J. Biol. Chem.* **259**, 10995–11000
27. Ruff, E., and Rizzino, A. (1986) *Biochem. Biophys. Res. Commun.* **138**, 714–719
28. Hall, P. K., and Roberts, R. C. (1978) *Biochem. J.* **171**, 27–38
29. Ganrot, P. O. (1966) *Clin. Chim. Acta* **14**, 493–501
30. Laemmli, U. K. (1970) *Nature* **227**, 680–685
31. Van Leuven, F., Cassiman, J.-J., and Van den Berghe, H. (1981) *J. Biol. Chem.* **256**, 9016–9022
32. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76**, 4350–4354
33. Huang, S. S., O'Grady, P., and Huang, J. S. (1988) *J. Biol. Chem.* **263**, 1535–1541
34. LaMarre, J., Wollenberg, G. K., Gonias, S. L., and Hayes, M. A. (1991) *Biochim. Biophys. Acta* **1091**, 197–204
35. Dennis, P. A., Saksela, O., Harpel, P., and Rifkin, D. B. (1989) *J. Biol. Chem.* **264**, 7210–7216
36. Danielpour, D., and Sporn, M. B. (1990) *J. Biol. Chem.* **265**, 6973–6977
37. LaMarre, J., Hayes, M. A., Wollenberg, G. K., Hussaini, I., Hall, S. W., and Gonis, S. L. (1991) *J. Clin. Invest.* **87**, 39–44
38. Coffey, R. J., Jr., Kost, L. J., Lyons, R. M., Moses, H. L., and La Russo, N. F. (1987) *J. Clin. Invest.* **80**, 750–757
39. Massagué, J., Cheifetz, S., Boyd, F. T., and Andres, J. L. (1990) *Ann. N. Y. Acad. Sci.* **593**, 59–72
40. Raines, E. W., Bowen-Pope, D. F., and Ross, R. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 3424–3428
41. Huang, J. S., Huang, S. S., and Deuel, T. F. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 342–346
42. Ronne, H., Anundi, H., Rask, L., and Peterson, P. A. (1979) *Biochem. Biophys. Res. Commun.* **87**, 330–336
43. Koo, P. H., and Stach, R. W. (1989) *J. Neurosci. Res.* **22**, 247–261
44. Borth, W., and Luger, T. A. (1989) *J. Biol. Chem.* **264**, 5818–5825
45. Matsuda, T., Hirano, T., Nagasawa, S., and Kishimoto, T. (1989) *J. Immunol.* **142**, 148–152
46. McCaffrey, T. A., Falcone, D. J., Brayont, C. F., Agarwal, L. A., Welth, F. G. P., and Weksker, B. B. (1989) *J. Cell Biol.* **109**, 441–448